The Toxicologist

Supplement to *Toxicological Sciences*

55th Annual Meeting and ToxExpo™

New Orleans, Louisiana

March 13–17, 2016

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 150, Issue 1
March 2016

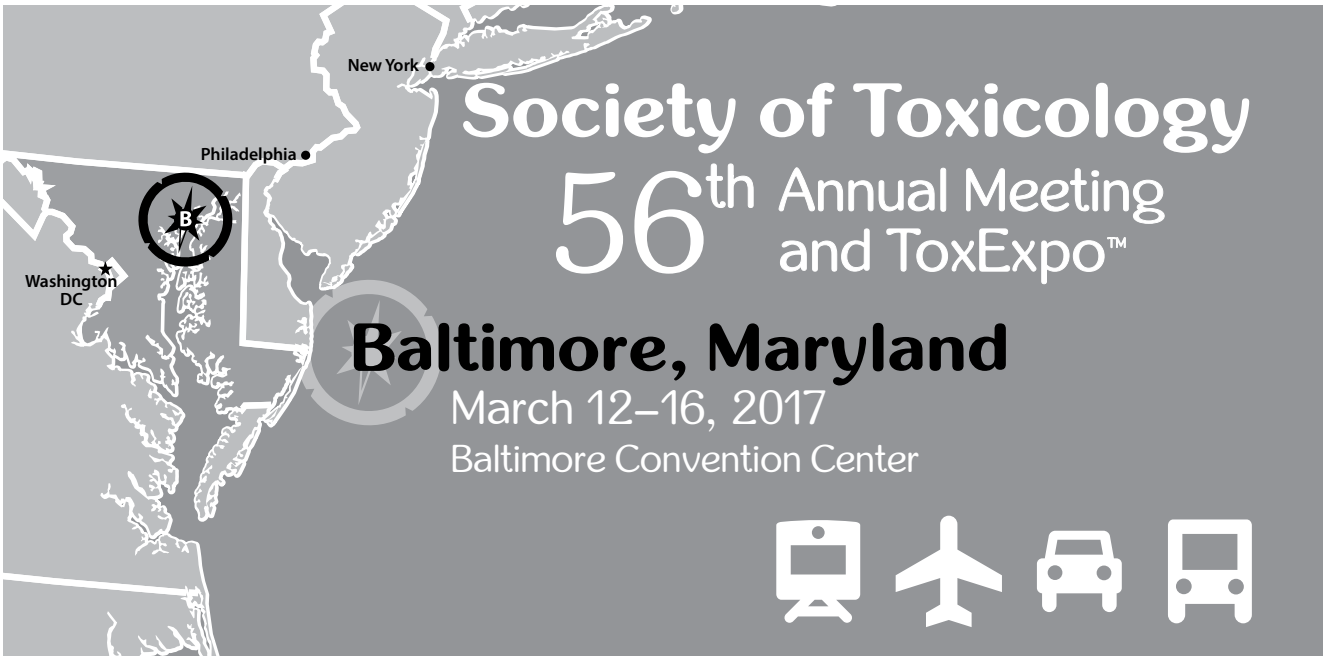
www.toxsci.oxfordjournals.org

The Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

www.toxicology.org



2017 Annual Meeting Session Proposal Deadline: April 30, 2016

Why Submit a Proposal?

1. To present new developments in toxicology
2. To provide attendees with an opportunity to learn about state-of-the-art technology and how it applies to toxicological research
3. To provide attendees with an opportunity to learn about the emerging fields and how they apply to toxicology

Session Types

Continuing Education—Emphasis on quality presentations of generally accepted, established knowledge in toxicology
Note: CE Courses will be held on Sunday.

Symposia—Cutting-edge science, new areas, concepts, or data

Workshops—State-of-the-art knowledge in toxicology

Roundtables—Controversial subjects

Historical Highlights—Review of a historical body of science that has impacted toxicology

Informational Sessions—Scientific planning or membership development

Education-Career Development Sessions—Sessions that provide the tools and resources to toxicologists that will enhance their professional and scientific development

Regional Interest—Central topics of relevance that describe public health and/or ecological problems of a particular region

Submit your proposal online at www.toxicology.org

The Toxicologist

Supplement to *Toxicological Sciences*

55th Annual Meeting and ToxExpo™

New Orleans, Louisiana

March 13–17, 2016

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 150, Issue 1
March 2016

www.toxsci.oxfordjournals.org

The Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

www.toxicology.org

Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 55th Annual Meeting of the Society of Toxicology, held at the New Orleans Ernest N. Morial Convention Center, March 13–17, 2016.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 603.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 629.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. Author names which are underlined in the author block indicate the author is a member of the Society of Toxicology. For example, J. Smith.

Scientific Session Types:

- CE Continuing Education Courses
- EC Education-Career Development Sessions
- FS Featured Sessions
- HH Historical Highlights Sessions
- IS Informational Sessions
- PL Platform Sessions
- PS Poster Sessions
- RI Regional Interest Sessions
- R Roundtable Sessions
- S Symposium Sessions
- W Workshop Sessions

The 2016 SOT Mobile Event App and Online Planner

The Mobile Event App and Online Planner are available via the SOT website and app marketplaces. These mobile tools enable you, the attendee, to engage with organizers, exhibitors, and each other, and to manage your time and maximize your experience during the Annual Meeting. You also can access some ePosters electronically via the Mobile Event App until May 11, 2016.

To cite a 2016 SOT Annual Meeting Abstract, please format as follows: *The Toxicologist*, Supplement to *Toxicological Sciences*, 150 (1), Abstract #__ , 2016, Title, First Author.

Copies of *The Toxicologist* are available at $40 each plus shipping ($15 shipping & handling in the USA and $50 for overseas shipments) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300 • Reston, VA 20190

www.toxicology.org

© 2016 Society of Toxicology

All text and graphics are © 2016 by the Society of Toxicology unless noted. For promotional use only. No advertising use is permitted.

This abstract book has been produced electronically by the Society of Toxicology. Every effort has been made to faithfully reproduce the abstracts as submitted. The author(s) of each abstract appearing in this publication is/are solely responsible for the content thereof; the publication of an article shall not constitute or be deemed to constitute any representation by the Society of Toxicology or its boards that the data presented therein are correct or are sufficient to support the conclusions reached or that the experiment design or methodology is adequate. Because of the rapid advances in the medical sciences, we recommend that independent verification of diagnoses and drug dosage be made.

1001 Basic Principles and Practices for Applying Epigenetics in Mechanistic Toxicology

S. D. McCullough[1] and R. N. Hines[2]. [1]*US EPA, Chapel Hill, NC and* [2]*US EPA, Research Triangle Park, NC.*

The genetic material of every organism exists within the context of regulatory networks that govern gene expression collectively called the epigenome. These epigenetic regulators, chromatin modifications, DNA methylation, and noncoding RNAs, act in concert to shape the way that cells, tissues, and organisms respond to their environment and toxicant exposure. Incorporating epigenetics into both *in vitro* and *in vivo* toxicological studies allows for a better understanding of the molecular events underlying the adverse health effects of toxicant exposure, improves our ability to identify vulnerable populations, and facilitates the identification of modifiable risk factors. The goal of this course is to provide toxicologists from a broad range of backgrounds with an overview of the epigenome and general considerations for designing experiments to examine the role of the epigenetics in their toxicological studies. This course will mention noncoding RNAs but focus primarily on participants gaining a fundamental understanding of the role of chromatin and DNA methylation in the regulation of gene expression. The principles and applications of basic experimental techniques, such as chromatin immunoprecipitation (ChIP) and DNA methylation analysis for evaluating epigenetic changes in toxicological studies, will also be discussed. This course will be of broad interest to investigators that are interested in integrating epigenetic approaches into their current or future toxicological studies.

1002 Advancing the Detection, Imaging, and Pitfalls in Monitoring Oxidative Stress in Health and Disease

M. B. Kadiiska[1] and R. P. Mason[2]. [1]*NIEHS/NIH, Research Triangle Park, NC and* [2]*NIEHS/NIH, Research Triangle Park, NC.*

Oxidative stress is recognized to play a role in the etiology of numerous diseases as well as in environmental exposures. Exploration of oxidative stress mechanisms is a field of ever-increasing attention, both in science and in commerce. The field is maturing and there is a great effort to study and understand biomarkers at both a chemical and enzymatic molecular-mechanism level. Since increases in oxidative stress are measured using biomarkers, the goal of this course is to convey the most up-to-date knowledge on biomarkers; present novel approaches and advanced methods that can be employed *in vivo* to measure, predict, and even prevent oxidative stress; and to discuss the methods and pitfalls for distinguishing oxidative stress from systemic toxicities, immunotoxicities and inflammation. Technologically advanced methods, including molecular magnetic resonance imaging, HPLC-MS, spectro(fluoro)metric assays, and immunoassays are potentially rich areas for innovation in systemic oxidative stress research. Consequently, the course outlines the most up-to-date developments in newly emerging methodologies that will enhance the understanding of oxidative stress mechanisms by measuring, detecting, and even imaging it *in vivo*. The panel of experts evaluates the advantages, applicability, and pitfalls of each method, discusses the most recent data on *in vivo* and *in situ* imaging of molecular free radical metabolites with emphasis on both the current state of technology, different areas of toxicology including immunotoxicology, crosstalks with the innate immune mediators, and likely future developments. Because the detection and understanding of oxidative stress could lead to better intervention strategies, output from the course will help identify the most useful applications for a given technique to detect oxidative stress *in vivo*. Attendees will leave the course with enhanced understanding that measurement of oxidative stress *in vivo* requires innovative, unconventional methodologies in combination with advanced technologies and often a multidisciplinary approach.

1003 Adverse Outcome Pathway (AOP) Development and Evaluation

S. Edwards[1] and A. Terron[2]. [1]*US EPA, Research Triangle Park, NC and* [2]*EFSA (European Food Safety Agency), Parma, Italy.*

The Adverse Outcome Pathway provides a construct for assembling mechanistic information at different levels of biological organization in a form designed to support regulatory decision making. In particular, it frames the link between molecular and cellular events that can be measured in high-throughput toxicity testing and the organism or population-level events that are commonly relevant in defining risk. Recognizing the importance of this emerging framework, the Organisation for Economic Co-operation and Development (OECD) launched a program to support the development, documentation, and consideration of AOPs by the international community in 2012. In 2014, a handbook was developed to guide users in the documentation and evaluation of AOPs and their entry into an official knowledgebase. The handbook draws on longstanding experience in consideration of mechanistic data (e.g., mode-of-action analysis) to inform risk assessment. To further assist users, a training program was developed by members of the OECD Extended Advisory Group to teach users the basic principles of AOP development and the best practices as outlined in the OECD AOP handbook. Training sessions began in early 2015, and this course will provide training for interested SOT scientists. Following this course, all participants will be familiar with the core principles of AOP development and assessment and the OECD efforts to support this effort. They will also know how the OECD guidance for AOP development has been implemented in the Wiki module of the AOP Knowledgebase. They will learn how to assemble and evaluate the evidence supporting the AOPs using established best practices from Mode of Action analysis. To reinforce the concepts, they will participate in a live demo where an AOP is developed from a training case study with their assistance and entered into the AOP-Wiki. The value of AOP development will be demonstrated via examples from the European Food Safety Agency and by considering integrated approaches to testing and assessment using the skin sensitization AOP, which was endorsed by the OECD in 2012.

1004 Contribution of Mitochondria to Drug-Induced Organ Toxicities

V. G. Desai[1] and Y. Will[2]. [1]*National Center for Toxicological Research, US FDA, Jefferson, AR and* [2]*Pfizer R&D, Groton, CT.*

Mitochondria generate more than 90% of energy essential for the cell. Impaired mitochondrial function, therefore, can affect virtually every tissue and organ in the living organism. Tissues with the highest energy needs, such as the heart, brain, liver, kidney, and skeletal muscle are particularly vulnerable to the defects in mitochondrial bioenergetics that can manifest into tissue-specific pathologies. A distinctive feature of mitochondria is that, besides the nucleus, these organelles contain their own genome (mitochondrial DNA). However, coordination between nuclear and mitochondrial genomes is crucial in regulating mitochondrial function. It is also becoming increasingly evident that mitochondria are a prime target of many therapeutic drugs and environmental toxins that can alter their function through different mechanisms, leading to cellular injury, resulting in organ toxicity, and, in the worst case, death. Additionally, mitochondria serve as an important player in the execution of apoptosis (programmed cell death), a process that serves as a major defense mechanism to remove unwanted and potentially dangerous cells. Collectively, these functions highlight a critical role of mitochondria in the life and death of the cell. This course will provide an in-depth overview of mitochondrial biology and different mechanisms in which drugs can affect mitochondrial function. Particular emphasis is given to mitochondrial toxicity causing heart, liver, and kidney injury. In addition, we will describe novel high-throughput *in vitro* screening technologies in isolated mitochondria and cell models to elucidate potential mitochondrial toxicity. Several other methodologies will also be discussed that can reveal the mitochondrial target(s) of drug toxicity in different organs. The utility and limitations of these approaches will also be described. This course concludes by providing the participants with in-depth knowledge of basic mitochondrial function and important insights into how subtle changes in mitochondrial activity can progress to overt pathology in tissues and help identify potential biomarkers of early stages of mitochondrial toxicity. Moreover, this course will present how preclinical data on mitochondrial toxicity can help in understanding toxicities in humans.

1005 Discovery and Validation of miRNA Biomarkers Bridging Preclinical and Clinical Toxicity: Lessons Learned from Hepatotoxicity

A. Harrill[1] and B. Chorley[2]. [1]*University of Arkansas for Medical Sciences, Little Rock, AR and* [2]*US EPA, Research Triangle Park, NC.*

Micro(mi)RNAs are small, noncoding RNAs that play an important role in the regulation of biological processes in cells. Owing to recent findings that: (1) miRNA sequences are highly conserved across species, (2) certain miRNAs exhibit tissue-specific expression, and (3) miRNA are highly stable in biological fluids, significant effort has been spent to identify miRNA biomarkers for a variety of toxicological pathologies. However, in addition to basic research and discovery efforts, significant effort must be spent on qualification of biomarkers with regards to specificity for the organ of interest and for the ability of a biomarker to detect comparable injury across species. A major effort is to use miRNA profiles as a "liquid biopsy" that can inform underlying tissue pathology. Thus, the goal of this course is to provide investigators with an overview of the

techniques and strategies necessary to progress a biomarker from the discovery stage to practical use in animal and human xenobiotic safety assessment. Well studied biomarkers, such as miR-122, will be used as case studies to demonstrate the path from nonclinical discovery to a validated clinical biomarker.

1006 Embryology and Developmental Toxicology Testing

J. M. DeSesso[1] and A. R. Scialli[2]. [1]*Exponent, Alexandria, VA and* [2]*Scialli Consulting LLC, Arlington, VA.*

Mammalian embryo-fetal development comprises a complex and carefully orchestrated set of activities that can be perturbed by maternal and environmental factors. Perturbations of developing offspring can result in no discernible effect, reduced fetal weights at term, increased prevalence of anatomical variations, congenital defects, and/or the demise of the offspring. This course will focus on preclinical species and will begin by providing an overview of mammalian development, including important gestational milestones, comparative interspecies timelines, and definitions of critical periods in development. Next we will discuss how this information has factored into the design of traditional preclinical studies. The presentation will conclude with a brief introduction to normal variability in some organ systems. This variability is the source of considerable controversy when interpreting traditional developmental toxicity safety tests. Succeeding presentations will discuss two organ systems that are the center of debate among scientists charged with extrapolating results found in safety assessments to potential human risk. The normal embryological development of the first organ system to function, the cardiovascular system, will be described with consideration of normal anatomical variations and nonadverse structural changes. The second organ system to be described is the skeletal system. Particular attention will be paid to its state of maturity at term in various species, the potential influence of maternal toxicity on skeletal maturation, and postnatal development of the skeleton throughout the lactation period. The final presentation will address the development of new testing methods that might be used to prioritize substances for testing or even to replace whole animal testing for developmental toxicity. The presentation will describe basic methods for whole embryo culture, embryonic stem cell test, and a Zebrafish assay, along with various proposed improvements in each. It will finish with some thoughts about integrating the results from multiple assays, and a survey of the regulatory landscape for these emerging methods. Information from the preceding presentations will provide the audience with an understanding of how the biological basis of prenatal developmental toxicity testing and the results of such tests should impact risk assessment and ultimately, the rationale for the design and use of drugs and chemicals that minimizes environmental impact and ensures human health.

1007 Next Generation Sequencing in Toxicogenomics

W. Tong[1] and J. Kleinjans[2]. [1]*National Center for Toxicological Research/US FDA, Jefferson, AR and* [2]*Maastricht University, Maastricht, The Netherlands.*

The purpose of toxicogenomics is to study the effects of chemical, biological and physical agents in biological systems at the molecular level and thereby elucidating the molecular mechanisms underlying the expression of toxicity. Recent technology developments in next-generation sequencing (NGS) have opened completely new possibilities for the deep characterization of molecular mechanisms of toxicity at various levels of cellular regulation providing information on substance-induced genomic variations, and on transcriptomic and epigenomic changes. We argue that these developments will strengthen our understanding of toxic mechanisms-of-action and ultimately lead to a systems-wide toxicity analysis thus enabling the development of safer drugs, industrial chemicals, consumer products and improved regulation. This course will discuss various NGS application for an enhanced understanding of underlying mechanisms of toxicity and potential utility in regulatory setting. The discussed topics include but are not limited to applicability of respective NGS platforms for analyzing mutational spectra, gene expression modifications, and epigenomic alterations induced by toxicants in a range of biological systems, compare their performance with standardized qPCR/microarray techniques, present use cases and highlight future challenges.

1008 Approaches to Investigate and Assess Risks Associated with Drug-Induced Liver Injury (DILI)

M. Otieno[1] and P. Watkins[2]. [1]*Janssen Pharmaceuticals, Spring House, PA and* [2]*UNC Institute for Drug Safety Sciences, Research Triangle Park, NC.*

Drug-induced liver injury (DILI) in the clinic is a major cause for drug attrition during development. DILI can be characterized as intrinsic or idiosyncratic. Properties of intrinsic DILI include a dose-response in presentation of injury that may be predicted by animal studies enabling application of safety thresholds and inclusion of liver injury biomarkers for clinical risk assessment. Idiosyncratic DILI (iDILI) is unpredictable and usually occurs following drug exposure in large populations e.g., during Phase III clinical trials or postmarketing. Given that this is a major cause for costly drug withdrawals, there has been significant effort in identifying properties that predispose some compounds to a high risk for iDILI. Both immune and nonimmune mechanisms are hypothesized to contribute to iDILI. This course will discuss DILI hazards that can be used to identify a compound's potential to cause DILI. A general overview and introduction of DILI will be provided, followed by a clinician's perspective on DILI focusing on presentation of DILI using examples of key withdrawals. Subsequent presentations will focus on established and emerging science on DILI hazard risks; this will include a presentation on the role of reactive metabolites (RM) and covalent binding in increasing risk for immune or nonimmune mediated DILI. A basic overview on mechanisms of RM formation, methods for detection, and mechanistic studies correlating covalent binding with DILI will be discussed. The relationship between dose, covalent binding thresholds, and DILI also will be addressed. This will be followed by a presentation on hepatic transporters and the role they play in DILI, either through delayed hepatotoxicity resulting from liver accumulation of parent/metabolites and/or inhibition of efflux of toxic bile acids. Mitochondrial toxicity also has been identified as a key hazard for DILI compounds; an overview of mitochondrial toxicity, its role in iDILI, and how interplay with hepatic transport inhibition may increase risk for DILI will be presented. The final presentation will introduce the concept of computational, systems pharmacology approaches integrating all of the mechanisms discussed by the previous speakers along with drug exposure, to put data from various sources into context.

1009 Exploring Chemical Space in the New Toxicity Testing Paradigm: From Data Curation to Computational Simulations

C. Tan[1] and D. Chang[2]. [1]*US EPA, Research Triangle Park, NC and* [2]*Chemical Computing Group, Inc., Montreal, QC, Canada.*

In an effort to determine mechanisms-of-action of toxicants known to induce adverse human health outcomes, traditional toxicity testing focuses on lengthy and costly *in vivo* animal studies on a chemical-by-chemical basis. In such a paradigm, there is no need to consider chemical space. The accelerated rate of production and distribution of new chemicals in the commercial market precludes such approaches today, and rather, high-throughput *in vitro* and *in silico* analyses are encouraged in order to rapidly screen hundreds to thousands of chemicals. The recent advancements in these high-throughput screening approaches, along with computational chemistry and cheminformatics, have provided toxicologists the foundation and opportunity to identify molecular fingerprints that impact each aspect of the source-to-outcome continuum for large amounts of chemicals. Inherent chemical structures and properties are key determinants of exposure potential (based on e.g., function and use), environmental persistence and transformation in environmental and biological systems, and toxicity potential. Computational approaches expand the chemical space through identification of chemicals with similar molecular descriptors capable of influencing these behaviors, thus allowing investigators to predict their toxicity or exposure potential, while foregoing actual testing. Attendees enrolled in this course will learn about the availability of different tools and vast numbers of resources to help them triage *in vitro* efforts, rationalize mode-of-action information, screen chemicals in a virtual world, and understand the issues of domain of applicability in toxicological studies. Investigators specializing in computational, molecular, and environmental toxicology, as well as those conducting high-throughput analysis for drug discovery or chemical screening, will experience the opportunity to address critical issues regarding evaluation of chemical space along the source-to-outcome continuum through comprehensive lectures and case studies.

1010 Genetics and Population Variability in Chemical Toxicity: The What, the How, and So What?

I. Rusyn[1] and B. A. Wetmore[2]. [1]*Texas A&M University, College Station, TX and* [2]*ScitoVation, LLC, Research Triangle Park, NC.*

The US EPA defines "variability" as "the range of toxic response or exposure—for example, the dose that might cause a toxic response can vary from one person to the next depending on factors such as genetic differences, preexisting medical conditions, etc." What are "genetic differences"? How do toxicologists and regulators estimate "population variability"? What new computational and experimental tools are available to substitute default "uncertainty factor" to account for variation in susceptibility among the members of the human population (i.e., interindividual variability)? This continuing education course is designed to review basics of genetics and demonstrate how appreciation for the role of genetic variability and novel experimental and *in silico* models can become key elements in human health assessments of chemicals. By superimposing the opportunities that are now afforded by sequencing technologies and novel experimental models and data onto the risk assessment paradigm, this course will be informative to the risk assessment practitioners and the toxicology research community, and increase the scientific impact of the fundamental toxicology studies. In addition, this course is directly responsive to the new SOT Strategic Plan (2015–2018) and the Central Challenge of shaping the future of toxicology in a changing scientific landscape. By using case studies of how the scientific disciplines of genetics and toxicology intertwine, this course will strengthen the impact and relevance of toxicology.

1011 Human Health Risk Assessment: A Case Study Application of Principles

J. C. Lipscomb[1] and M. E. Meek[2]. [1]*US EPA, Cincinnati, OH and* [2]*University of Ottawa, Ottawa, ON, Canada.*

This advanced, case study application course will build on course content previously presented and archived by the Society of Toxicology through CE courses presented in 2013 (Basic Principles of Human Risk Assessment) and 2014 (Methodologies in Human Health Risk Assessment). In this course, real world examples from publicly available, peer reviewed, completed risk assessments will be used as teaching aids. Course modules will be organized according to the four components of the Risk Assessment Paradigm: Hazard Characterization, Dose-Response Assessment, Exposure Assessment, and Risk Characterization. The Hazard Characterization component will consist of a guided case study based evaluation of the strength and consistency of available hazard data culminating in a weight of evidence synthesis of hazard information; Dose-response information including default (allometric scaling), pharmacokinetic approaches including a live benchmark dose application from completed assessments will be presented and discussed; Information documenting actual (measured) exposure and/or data useful in determining a default measure of exposure will be presented and discussed; Risk Characterization will demonstrate the development of drinking water maximum contaminant levels, maximum contaminant level-goals, reference values, and cancer slope factors; as well as methods to estimate risk at a given contaminant level. The course booklet will contain a worksheet on the risk assessment examples, to be completed during the class. Unique to this course, students will be provided a risk assessment problem consisting of fundamental environmental contamination levels and original publications describing toxicity studies and will be asked to characterize the hazard, estimate exposures via soil and water, develop measures of toxic potency, and develop risk values for a hypothetical environmental contaminant. The results will be provided through an open access "drop box" type application.

1012 Unique Approaches to Safety Assessment of Gene, Cell, and Nucleic Acid-Based Therapies

T. MacLachlan[1] and J. Cavagnaro[2]. [1]*Novartis, Cambridge, MA and* [2]*AccessBio, Boyce, VA.*

The platforms used for therapeutic treatment of disease have been greatly expanding over the last decade beyond the standard small molecule approaches and the now widespread use of proteins and monoclonal antibodies. The prospect of gene therapy began several decades ago with the promise that misfunctioning genes could be simply replaced, but was stunted in its growth with several notable safety events in the clinic. Now gene therapy is making a furious comeback, with several industry and academic groups employing various technologies and racing to catch up. Cell therapy has experienced similar peaks and valleys in interest, with stem cells touted as a platform able to replace entire damaged organ systems. Multiple variants of what one would call a cell therapy now are in development ranging for treatment with fully differentiated somatic cells to naïve cells able to grow and differentiate *in vivo*. A combination of gene and cell therapy approaches is used in the widely popular T cell immunotherapy approaches for cancer treatment where cells are modified *ex vivo* to target tumors after reintroduction to the patient. Considering the potency of T cells it is not surprising that safety concerns have limited their target profile. Finally, the concept of knocking down expression of gene expression has gained significant momentum with the introduction of therapeutic RNA interference and most recently with gene editing via a variety of methods. All of these "advanced therapy" platforms require very unique approaches outside of the standards defined by internationally accepted guidance for preclinical safety assessment. Not only does the biopharmaceutical mantra of "case by case" apply, but standard issues such as immunogenicity, tumorigenicity and appropriate animal models take on new meaning when applied to these therapies. With start-up companies being formed and larger pharmaceutical companies investing heavily in these therapeutic areas, there is a growing need for toxicologists to be familiar with these platforms. This course will aim to introduce the audience to each of these four general categories of advanced therapies as well as provide a regulatory perspective on these modalities and highlight where the standard approaches for safety assessment either do not apply or require unique application.

1013 Zebrafish As a Tool in Toxicology and Drug Discovery Screening

M. Behl[1] and A. Muriana[2]. [1]*National Toxicology Program/NIEHS, Research Triangle Park, NC and* [2]*BBD BioPhenix S.L.-Biobide, San Sebastián - Donostia, Spain.*

The zebrafish is a powerful tool to screen compounds, especially for early development since it is a vertebrate, has a high genetic homology with humans, and the assays can be automated to provide relatively high-throughput along with high-content analysis. Adult and developing zebrafish are increasingly being used to assess development, gene expression, and behavior to screen and prioritize compounds for toxicity testing and safety evaluation. Specifically, the zebrafish has proven extremely relevant in the evaluation of acute toxicity and developmental toxicity, as well as organ-specific toxicities, including cardiotoxicity, hepatotoxicity, and neurotoxicity. The purpose of this course is to bring together experts from the pharmaceutical industry, CROs, academia, and the Government to provide insight on the current developments in the use of zebrafish in the field of toxicology and drug safety and efficacy assessment, and to highlight some ongoing challenges in the field. The course will begin with important basics of zebrafish biology such as selection of strains, husbandry, dose-response and time course, and will highlight its application in toxicology. The second talk will focus on the recent advancements of zebrafish as a tool to assess developmental toxicity. The third talk will focus on the systematic optimization of variables for efficient screening, and tools for data management, visualization and analysis. Speaker four will highlight the use of zebrafish in drug discovery as a rapid and cost effective method to screen for a number of new molecular entities entering clinical phases. Finally, speaker five will build upon the information presented by the previous speakers to shed light on the utility in screening of drugs for efficacy and safety, and ultimately for acceptance of data for regulatory purposes. The course will address critical questions on the status of this model in hazard identification and risk assessment for environmental toxicants including progress in the field, loopholes, and data-gaps, novel upcoming developments, and the impact of this model on toxicology research in the 21st century, as well as the impact on safety assessment of drugs.

1014a Opening the Black Box: Understanding the Molecular Mechanisms of Developmental Toxicity

B. McIntyre[1] and J. Gamse[2]. [1]*NIEHS, Research Triangle Park, NC and* [2]*Bristol-Myers Squibb, New Brunswick, NJ.*

The development of an organism from egg to adult is a complex series of interlocked events, which depends on precise coordination in time and space. When xenobiotic agents interfere with development, they can alter cellular growth and differentiation, leading to permanent changes in tissue/organ structure and function, i.e., developmental malformations. Over the past 40+ years, developmental toxicologists have strived to understand the mechanisms of action resulting in these lesions. With the advent of novel molecular tools, an enhanced understanding of developmental biology, and new model systems, great progress has recently been made in deciphering some of the fundamental drivers of altered development. In this session, we will explore recent findings in developmental toxicology that have begun to link teratogens with their potential mechanisms of action. The first speaker uses a zebrafish cardiovascular model to explore the role of the G-protein-coupled estro-

gen receptor on heart rate. In the second presentation, the speaker describes a novel mechanism for arsenic disruption in TGFB-SMAD signal transduction during formation of heart valves and coronary vessels. The third speaker demonstrates how altered TGF-beta and FGF signaling impact the development of the forebrain. Using an *in vitro* murine limb bud culture system and classic histone deacetylase inhibitors, the fourth speaker reveals the role of protein acetylation in the action of some developmental toxicants. Finally, using thalidomide and its analogs, the fifth speaker presents compelling data on the mechanism of thalidomide action in phocomelia. These speakers, who come from a mix of academic and industry backgrounds, will demonstrate how understanding the mechanisms underlying developmental toxicology allows for the prediction of class effects, the discovery of subtle but important developmental changes, and the design of more informative *in vitro* and *in vivo* methods for detecting teratogenicity.

S 1014b Acute Estrogen Exposure Increases Heart Rate via a G-Protein-Coupled Estrogen Receptor Mechanism in Zebrafish

D. Gorelick. *University of Alabama, Birmingham, AL*. Sponsor: B. McIntyre.

Embryonic blood flow influences the development of the heart, vasculature and hematopoetic stem cells. Thus, the establishment of normal embryonic heart rate is thought to be essential for proper development and function of the cardiovascular system. Whether estrogenic environmental endocrine disruptors modulate heart rate is not well understood. Here we demonstrate that exposure to exogenous estrogens causes an acute increase in heart rate in zebrafish embryos via a G protein-coupled estrogen receptor (GPER). Pharmacological inhibition of estradiol synthesis caused a reduction in heart rate that was rescued by: 1) exogenous estradiol, 2) a membrane-impermeable estradiol, and 3) a specific GPER agonist. Exposure to a GPER agonist alone increased heart rate, while co-administration of a GPER antagonist blocked the increase in heart rate. To probe this signaling pathway further, we generated homozygous GPER mutant embryos, which exhibited a lower basal heart rate compared to wildtype embryos, suggesting that endogenous estrogens increase heart rate. Consistent with this hypothesis, we identified endogenous estradiol in embryos using a new mass spectrometry assay we developed. Using histology and genetic rescue techniques, we are working to identify the cell type(s) in which estrogens act to increase heart rate acutely. We are also testing whether GPER mutants exhibit abnormal cardiovascular development in embryos and abnormal heart function in adult animals. Thus, studying the developmental toxicity of estrogenic endocrine disrupting compounds revealed a new physiological mechanism by which environmental and endogenous estrogens modulate heart rate, with implications for embryonic cardiovascular development and adult heart function.

S 1015 Fetal Arsenic Exposure Disrupts TGFß2-SMAD Signaling and Developmental EMT

T. Camenisch. *University of Arizona, Tucson, AZ.*

There is limited understanding on the molecular basis of developmental toxicity by arsenic. We reveal that TGFß2 signaling is a target of arsenic and as such disrupts key developmental processes. TGFß2 is a key growth factor regulating epithelial to mesenchymal transition (EMT) in several tissue compartments during embryogenesis. TGFß2 triggers cardiac progenitor cells to transform into mesenchymal cells and give rise to the cellular components of coronary vessels as well as cells of the heart valves. TGFß signaling is dependent on a dynamic on and off switch in Smad activity. Chronic arsenic exposure disrupts Smad activation leading to deficits in TGFß2 mediated EMT. In addition, TGFß2 induced Smad2/3 nuclear shuttling is attenuated during short exposures to arsenic independent of Smad phosphorylation or nuclear importation. This depletion in nuclear Smad is restored by knocking-down Smad specific exportins, Exportin 4 or CRM1, suggesting that arsenic augments Smad2/3 nuclear exportation. Zinc supplementation reverses the disruption in Smad2/3 nuclear translocation by arsenic revealing a novel mechanism. This coincides with Zinc rescue of arsenic mediated deficits in cardiac EMT. Thus, transformation and invasive cell motility of EMT derived mesenchyme are protected from arsenic toxicity by zinc supplementation. These findings establish a novel mechanism for arsenic disruption in TGFß-Smad signal transduction during development.

S 1016 TGFß and FGF Inhibition Elucidates Mechanisms Controlling the Formation of the Diencephalon

J. Gamse. *Bristol-Myers Squibb, New Brunswick, NJ.*

Inappropriate activation of the TGFß and FGF signaling pathways occurs in many disease states, including fibrosis and cancer, so receptors for both pathways have been targets for pharmaceutical inhibition. However, both pathways also play central roles in the development of the brain, particularly for the dorsal habenulae. These bilateral brain nuclei play a central role in both anxiety modulation and addiction. The habenulae are divided into medial and lateral subnuclei with distinct populations of neurons. Allocation of neurons to the subnuclei is controlled by the timing of the neuron's birth, with early-born neurons preferentially contributing to the medial subnucleus and later-born neurons to the lateral subnucleus. TGFß signaling contributes to the timing of habenular neurogenesis, but must use an intermediate signal, as TGFß ligands and receptors are no longer expressed when neurons are being produced. We find that FGF signaling is the intermediate signal. Using small-molecule inhibitors and activators of FGF signaling, we show that habenular neurogenesis is altered in a dose-dependent manner. Further, we identify that FGF signaling regulates expression of the cell-cycle-dependent kinase inhibitor (CDKI) kip2, providing a mechanism for FGF to control cell cycle exit and promote neuronal birth. Finally, a small molecule inhibitor of TGFß receptors demonstrates its regulation of FGF activity. We propose that FGF signaling serves as a key regulator of neurogenesis in the habenular nuclei, integrating TGFß signaling with control of cell cycle in neuronal precursors. Moreover, great care should be taken to evaluate brain development during preclinical testing of compounds affecting these pathways.

S 1017 The Role of Histone Deacetylase Inhibition in Mediating the Effects of Developmental Toxicants on Limb Development

B. Hales. *McGill University, Montreal, QC, Canada.*

Histone deacetylases (HDACs) play a major role in chromatin remodeling, the regulation of gene expression and cell signaling. Several inhibitors of these enzymes have been shown to increase the incidence of birth defects. However, the mechanisms leading to their teratogenicity and the role of each class of HDACs during normal development are not clear. Valproic acid, a class I and II HDAC inhibitor and an anticonvulsant drug, induces neural tube defects and skeletal malformations *in vivo*. In the *in vitro* murine limb bud culture system valproic acid causes a significant concentration-dependent increase in limb abnormalities and dysregulates the expression of Sox9 and Runx2, two transcription factors that are key in chondrogenesis and osteogenesis in the limb. Valpromide, a close analog of valproic acid and a weak *in vivo* teratogen, does not inhibit HDAC activity and has little effect on either limb morphology or the expression of marker genes. Thus, the effects of valproic acid are correlated with its HDAC inhibitory activity. Subsequent studies on the class-specificity of the effects of HDAC inhibitors in the limb bud culture system have revealed that inhibition of class II HDACs has a minimal impact on limb differentiation; in contrast, inhibition of class I or III HDACs is associated with severe developmental toxicity. Together, these studies indicate that dysregulation of the acetylation of specific target proteins may be important in mediating the action of some developmental toxicants. Supported by CIHR.

S 1018 Elucidation of the Mechanism of Action of Thalidomide and IMiD Compounds Gives Novel Insights into Their Pleiotropic Effects

R. Chopra. *Celgene, Summit, NJ*. Sponsor: B. McIntyre.

Thalidomide and its analogues, the IMiD compounds Lenalidomide and Pomalidomide have been transformational for the pharmaceutical industry; the thalidomide tragedy altered our perceptions of safety monitoring and transformed the drug regulatory environment. Subsequently the IMiD compounds have now established roles in multiple myeloma, mantle cell lymphoma, and myelodysplastic syndrome. The precise molecular mechanism of action of these drugs, however, has been unknown. A potential unifying key to unraveling the actions of the IMiDs began with the relatively recent observation that thalidomide caused phocomelia birth defects by binding to cereblon (CRBN), part of a complex with E-3 ubiquitin ligase activity. We solved the structures of CRBN in the apo state and with thalidomide or Pomalidomide and this has given us insights as to why the rodent model did not give the limb malformations seen in other species. In order to identify the amino acids responsible for this difference, we tested a series of human/mouse CRBN chimeric proteins for their ability to confer Lenalidomide-induced activity. Substitution of amino acids in human CRBN with the

corresponding amino acid in mouse CRBN revealed only one substitution, V387I (human CRBN isoform 2), that disrupted the Lenalidomide-responsiveness of human CRBN. Remarkably, substitution of the isoleucine at this position in mouse CRBN for the human valine was sufficient to confer Lenalidomide-induced activity in mouse cells. Taken together our data provide important mechanistic insights into the activity of IMiD drugs by enabling the development of novel agents that can modulate CRBN activity in different cellular contexts to gain a broad range of therapeutic activities.

W 1019 Dietary Exposures to Heterocyclic Amines As a Potential Risk Factor for Neurological Disease

J. Cannon[1] and K. Turteltaub[2]. [1]*Purdue University, West Lafayette, IN and* [2]*Lawrence Livermore National Laboratory, Livermore, CA.*

Neurodegenerative diseases, such as Parkinson's Disease (PD), have been repeatedly linked to diverse risk factors, including genetics and environmental exposures such as pesticides, solvents, and a variety of heavy metals. Further, epidemiological and genetic data suggest that no single toxicant class or genetic mutation is a major cause of the idiopathic forms of this disease. Aside from coffee (caffeine), dietary factors have not been examined to the same extent as environmental toxicants as potential etiological factors in PD. Heterocyclic aromatic amines (HAAs) may be encountered in a variety of foods, notably cooked meats and drinks. High-temperature cooking and resultant charring can produce several toxic compounds, including HAAs. There is limited knowledge on the neurological effects of HAAs. However, multiple HAAs affect dopamine metabolism, and specific HAAs have been linked to essential tremor and, more recently, PD. This workshop will focus on newly emerging data suggesting a potential role of HAAs in neurological diseases, including PD. A diverse group of experts have been assembled to address this especially timely topic. In fact, recent publications from the speakers' laboratories have shown that HAA blood levels are associated with essential tremor and PD and that *in vitro* and *in vivo* treatments are selectively toxic to dopaminergic neurons. Speaker expertise includes clinical studies (neurology, Elan Louis), *in vitro* and *in vivo* modeling (neurotoxicology, Jason Cannon), analytical/transport (PhIP quantification/pharmacokinetic analysis in biological fluids and tissue, Kenneth Turteltaub), and biomarkers (exposure and adduct formation, Robert Turesky). The session will begin with a brief introduction of the key findings to date and gaps in the literature. In each of the talks, the speaker will relate how doses in their cellular and animal studies relate to typical human exposures. The first speaker will discuss the association of HAA blood levels in humans with the essential tremor and PD. The second speaker will discuss the utilization of novel blood-brain-barrier models to predict HAA entry into the brain. This speaker will also discuss how this model system, in conjunction with significant pharmacokinetic data collected in both human studies and animal models, might be used in translational studies. The third speaker will discuss data on selective dopaminergic toxicity of specific HAAs. While the relationship between HAAs and essential tremor is much more established, published and preliminary data now suggests that more detailed studies in PD and PD models are needed. Multiple brain systems are affected in PD; however, loss of dopaminergic neurons in the substantia nigra or changes in their function are a major contributor to the characteristic motor phenotypes. Thus, initial studies in primary culture focus on determining if dopamine neurons exhibit heightened sensitivity to specific HAAs, compared to gamma-aminobutyric acid (GABA)-ergic neurons (spared in PD). Emerging data on ET pathophysiology suggests that cerebellar abnormalities may important. Thus, ET-relevant pathologies that should also be investigated will be discussed. The fourth speaker will broadly discuss mechanisms of toxicity and innovative biomarker studies being conducted in humans and animal models. Here, how actual human exposures and systemic biomarkers relate to laboratory animal studies will be discussed. Significant time will be allotted for a critical evaluation of the presentations to identify key gaps in the literature. Given that the literature on HAAs is focused on genotoxicity, the discussion will emphasize the following key gaps: 1) What is the potential overlap with key neurotoxic mechanisms of action? and 2) How should such mechanisms be explored? The limited identification of causative factors of neurological diseases provides an impetus for this timely workshop.

W 1020 Relationship between Blood Harmane Levels and Neurodegenerative Disease

E. Louis. *Yale School of Medicine, New Haven, CT*. Sponsor: J. Cannon.

Total time = 30 minutes (talk + questions). The talk will highlight accelerating work over the past 10 years that has increasingly linked heterocyclic amine exposure with several neurological diseases, including essential tremor (ET) and Parkinson's disease (PD). The talk will focus on human subjects research, which has explored links between both blood and brain levels of the ß-carboline (subclass of heterocyclic amines) harmane and neurological diseases in patient populations. Previous studies in animals have found harmane and other alkaloids to be potent tremor-producing neurotoxins, but these studies have brought the link directly into the clinical arena. These compounds are present in a variety of animal and plant-derived foods. Our data on the relationship between blood as well as brain levels and neurological disorders suggests that in the future both human epidemiology and basic science neurotoxicology experiments are essential to understanding the potential pathogenic role that exposures may have in neurodegenerative diseases. Dr. Louis's clinical research and insights are critical to the workshop. He is a neurologist with clinical expertise in movement disorders (including ET and PD) and he has an extensive research track record in exploring etiological factors. The presentation of human data is critical to the translational focus of this workshop. Dr. Louis's expertise, as a clinician and epidemiologist is also critical to moderating the discussion.

W 1021 Novel Blood-Brain Barrier Models to Assess PhIP Transport into Brain

K. Turteltaub. *Lawrence Livermore National Laboratory, Livermore, CA.*

Total time = 30 minutes (talk + questions). PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) is a heterocyclic amine found in cooked meat, which has been extensively studied for its carcinogenicity. At high doses, PhIP has also been shown to cause tremors in rodents and damage neurons in the brain. Recent studies suggest PhIP affects dopaminergic neurons and cause PD like symptoms. One critical question that is currently a key gap in the literature related to neurotoxicity potential is how well PhIP crosses the blood brain barrier at doses relevant for human dietary exposure? We have developed an *in vitro* model and investigated whether PhIP and its key metabolites cross the barrier at cancer bioassay levels that bear relevance to dietary exposures. Our results also suggest PhIP is not metabolized by the cells making up the blood brain barrier but PhIP and N-OH PhIP do cross the barrier at both high and low doses. Various inhibitors suggest the mechanism for transport. Our data suggests PhIP passively diffuses across the membrane but may be actively effluxed by the ABS pumps. We therefore found that PhIP and its metabolite do cross the blood brain barrier in a dose dependent manner and is thus capable of damaging neurons in the brain. Our data is expected to be useful for translational studies. Specifically, our novel BBB transport model can be used to describe an initial relationship between systemic and brain PhIP concentrations. Given that our group has assessed PhIP and PhIP metabolite levels in both human and animal studies, we will discuss how this new BBB transport system will, in future translational studies provide a framework for estimating human brain levels.

W 1022 PhIP Exposure and Dopaminergic Neuron Toxicity

J. Cannon. *Purdue University, West Lafayette, IN.*

Total time = 30 minutes (talk + questions). Dopaminergic neurons are selectively lesioned in Parkinson's disease. Our data indicates that PhIP is selectively toxic to dopaminergic neurons in primary cell culture experiments, where both dopaminergic and nondopamiergic cell loss and neurite retraction were assessed. In this system, GABAergic neurons were spared at doses that were toxic to dopaminergic neurons. Neuroprotection studies suggest that the toxicity is primarily mediated through oxidative stress. Further, metabolite toxicity suggests that nitrenium ion formation may be of key importance to neurotoxicity. Data from ongoing *in vivo* studies will also be presented along with efforts focused on determining mechanisms of neurotoxicity. Our preliminary data suggests that PhIP is also toxic to nigral dopamine neurons *in vivo*, with evidence of oxidative damage in nigral DA neurons after systemic exposure. Thus, our data suggests that PhIP should be considered as a putative PD-related toxicant.

1023 Heterocyclic Aromatic Amine Exposure: Biomarker Studies from Rodents to Humans

R. Turesky. *University of Minnesota, Minneapolis, MN.* Sponsor: J. Cannon.

Total time = 30 minutes (talk + questions). PhIP, 2-amino-3,8-dimethylmidazo[4,5-f]quinoxaline (MeIQx), are prominent heterocyclic aromatic amines (HAAs) formed in well-done cooked meat and tobacco smoke. 2-Amino-9H-pyrido[2,3-b]indole is the predominant HAA formed during tobacco combustion. HAAs undergo metabolic activation by cytochrome P450s, to form genotoxic N-hydroxylated metabolites, which react with DNA to form covalent adducts. If not repaired, adducts can lead to mutations. Long-term HAA exposure and their DNA adducts may be associated with carcinogenicity or neurodegeneration. N-hydroxylated metabolites of HAAs also react to form adducts with proteins and N-hydroxylated HAAs undergo redox cycling to generate reactive oxygen species that induce oxidative damage to DNA and proteins, also leading to toxicity. The catalytic efficiency of human P450 1A2, the major P450 involved in bioactivation of HAAs, is superior to rodent orthologues, commonly used in subchronic and chronic bioassays. Thus, genotoxicity and toxicity data generated in rodent models may underestimate the health risk posed by HAAs. The major pathways of metabolism of HAAs are known, and a number of HAA metabolites have been identified in urine of omnivores. However, the most promising long-term biomarker of exposure to HAAs is the accrual of PhIP in hair. The noncovalent binding of PhIP is largely driven by the level of pigmentation of the hair. This biomarker is being implemented in molecular epidemiology studies designed to address the role of PhIP in dietary-linked cancers, but could be employed in studies designed to assess the role of exposure of this HAA in neurodegenerative diseases.

1024 Mitochondria As the Central Target of Environmental Contaminants, Pharmaceutical Agents, and Toxicants: Mechanisms of Toxicity and Disease

R. Franco[1] and J. N. Meyer[2]. [1]*University of Nebraska-Lincoln, Lincoln, NE and* [2]*Duke University, Durham, NC.*

Mitochondrial dysfunction is widely recognized as a central component in the etiology of human diseases such as cancer and neurodegeneration, as well as in numerous other rare disorders. Furthermore, many pharmaceuticals, drugs, and contaminants have been identified as previously unrecognized mitochondrial toxicants. However, the exact molecular mechanisms by which mitochondrial dysfunction regulates disease progression are poorly understood. In this workshop we will review novel research findings regarding the causative role of mitochondria dysfunction and the resultant alterations in cellular bioenergetics, redox signaling, and DNA-damage response to disease progression induced by environmental contaminants, pharmaceutical agents, and toxicants (substance abuse). We will also discuss advances made by the pharmaceutical industry in investigating mitochondrial toxicities of drugs, and present novel toxicological approaches for metabolomics, high-throughput analysis of bioenergetics, and mitochondrial toxicity, as well as the importance of these approaches for the identification of novel mechanisms of disease progression and environmental and pharmacological/drug abuse risk factors. This topic will be of great importance to basic researchers, graduate students, postdoctoral trainees, and academics across disciplines as well as risk assessors, regulators, and individuals within distinct Specialty Sections.

1025 Mitochondrial Redox Signaling Regulates Cellular Metabolism and Drives Cancer Progression

M. Bonini. *College of Medicine. University of Illinois at Chicago, Chicago, IL.* Sponsor: R. Franco.

Mitochondria are critical cell signaling hubs that impact cellular metabolism by controlling both oxygen disposition and ATP production. Our results have contributed to establish that the switch of the normal oxidative cellular metabolism to glycolysis (aka. Warburg effect) results from a mitochondria-driven activation of glycolysis initiated by changes in the mitochondrial redox proteome. Specifically, we found that the upregulation of mitochondrial superoxide dismutase (SOD2) enhances H2O2 levels in mitochondria and increases the outflow of H2O2 to the cytosol. H2O2 activates AMP-activated kinase (AMPK) via oxidation of calmodulin kinase II and this effect is required and sufficient to sustain ATP production and NADPH homeostasis in breast cancer cells. More recently, we have also found that toxicants that redirect oxygen utilization from ATP production to H2O2 induce glycolytic metabolism priming cells to oncogenic transformation. One such insult is ethanol, whose heavy consumption increases cancer risk. Ethanol enhances SOD2 expression thereby inducing the Warburg effect. With focus on the cross talks between chronic exposures, inflammation and cancer I will develop the concept that changes in the mitochondrial redox state drive critical metabolic changes in the cell that enable cancer onset and progression.

1026 Energy Metabolism, Redox Homeostasis, and Dopaminergic Cell Death Induced by Gene-Environment Interactions

R. Franco. *University of Nebraska-Lincoln, Lincoln, NE.*

While aging is the major risk factor in Parkinson's disease (PD), its multi-factorial etiology also includes genetic alterations and exposure to environmental agents. Environmental toxicants are unlikely the single cause of PD. However, understanding the cellular dysfunction induced by these agents will reveal the toxic mechanisms by which they interact with other risk factors. The loss of dopaminergic cells in PD is associated with mitochondrial dysfunction, but the exact contribution of cellular metabolism, bioenergetics and oxidative stress, to dopaminergic cell death is unclear. We have developed an integrated nuclear magnetic resonance (NMR)/mass spectrometry (MS) metabolomics platform and metabolic flux analysis, and identified specific metabolic alterations induced by the mitochondrial/environmental toxicants. We have revealed that alterations in carbon metabolism are tightly linked to oxidative stress and cell death progression. For example, impairment of the TCA cycle and accumulation of citrate induced b y paraquat, inhibits glycolysis and directs carbon metabolism to the pentose phosphate pathway (PPP) to increase paraquat's redox cycling and oxidative stress. Both molecular and pharmacological modulation of the PPP directly correlates with the toxicity of paraquat. Furthermore, overexpression of a-synuclein (wild type or its mutant form A53T) synergize with environmentally relevant doses of paraquat to upregulate glucose transport and the PPP to trigger dopaminergic cell death. These and other findings presented will highlight the contribution that alterations in cellular metabolism have on dopaminergic cell death induced by mitochondrial dysfunction linked to gene-environment interactions.

1027 Gene-Environment Interactions in Mitochondrial Diseases

S. Chan. *Medical University of South Carolina, Charleston, SC.*

The etiology of mitochondrial diseases can be complex. The mitochondrion is under the control of both the nuclear and the mitochondrial genome. Even with the same genetic mutation, different ages of onset and disease phenotypes may result. Numerous environmental toxicants and pharmacological agents also impair mitochondrial function. We have validated the zebrafish as an excellent vertebrate model organism for toxicology, development and lifespan studies. In addition, we have also developed new tools and techniques for measuring mitochondrial parameters in the zebrafish, where we have taken advantage of its unique qualities such as their high fecundity and small size of embryos for high-throughput *in vivo* studies. Furthermore, the transparency of the embryo allows us to measure mitochondrial dynamics in different tissues. Our data show that the high energy tissues (nervous and cardiovascular tissues) are most susceptible to mitochondrial dysfunction caused by genetic manipulation, or exposures to mitochondrial toxicants, which recapitulates the vulnerable tissues of human patients with mitochondrial disease. We hypothesize that the variable phenotypic spectrum of genetic mitochondrial disease is due to exposures to mitochondrial toxicants, and with these new models and tools we are working on the gene-environment interactions involved in onset and progression of disease. Furthermore, these new *in vivo* models are being used to screen for compounds that rescue phenotypic and mitochondrial defects, for use in human mitochondrial diseases.

1028 Genetic and Developmental Stage Sensitivity to Mitochondrial Toxicity

J. N. Meyer. *Duke University, Durham, NC.*

Many mitochondrial homeostasis genes are human disease genes, and deficiencies in these genes may sensitize to exposures. The dramatic changes that occur in mitochondria during development also suggests that the same exposure may cause different toxicity at different stages. Mitochondrial DNA (mtDNA) lacks some repair pathways that are present in the nucleus such that damage formed after exposure to important genotoxins such as ultraviolet C (UVC) radiation and some polycyclic aromatic hydrocarbons is not repaired in mtDNA. Therefore, this damage is persistent, although it is slowly removed in a process dependent at

least in part on autophagy. Using the nematode model Caenorhabditis elegans, we found that UVC-induced photodimers result in lower levels of mtDNA-encoded mRNAs, decreased ATP levels, decreased oxygen consumption, larval developmental arrest, and dopaminergic neurodegeneration. Furthermore, mutations in mitochondrial fusion, fission and autophagy genes exacerbate the UVC-induced larval arrest, and also sensitize nematodes to other environmental and pharmaceutical agents including arsenic, cisplatin, rotenone, and paraquat. In some cases, developmental exposures result in lifelong deficits in mitochondrial function. Metabolomic data suggest that this occurs as a result of persistent diversion of metabolic intermediates towards protection against mitochondrial oxidative stress, a model that we are currently testing. Overall, our results suggest a potent gene-environment interaction in which the effects of mtDNA damage are exacerbated by decreased mitochondrial homeostasis.

W 1029 Drug-Induced Mitochondrial Toxicity—A Decade of Assay Development, a Decade of Learning

Y. Will. *Pfizer Inc, Groton, CT.*

Drug-induced mitochondrial toxicity leads to a variety of organ toxicities, most noticeable drug induced liver injury, but also cardiac, muscle and CNS toxicity, and has contributed to market withdrawals and black box warnings. Animal models used in preclinical safety assessment fail to reveal mitochondrial toxicity. The withdrawal of the two blockbusters troglitazone and cerivastatin prompted the pharmaceutical industry about implementing a screening paradigm, as in both cases, mitochondrial toxicity was thought to at least in part contribute to the observed liver toxicity of troglitazone and the rhabdomyelisis (breakdown of muscle tissue) induced by cerivastatin. Whereas mitochondrial toxicity had been studied in academic setting for over 25 years, the lack of high throughput applicable assays provides a challenge. I will introduce currently available technologies that can help to elucidate potential mitochondrial toxicity. This includes screens using isolated mitochondria and cell models that truly reveal mitochondrial toxicity. In addition, several methodologies will be discussed that can elucidate the target(s) of such toxicity. Several drug classes, such as glitazones, statins, antidepressants, NSAIDs, antibiotics and antivirals will be showcased to demonstrate the utility and or predicitivity of the individual assays.

W 1030a Nanotoxicology and Ocular Drug Delivery: One Size Does Not Fit All

C. J. Somps[1] and D. A. Fox (OTSS member)[2]. [1]*Pfizer, Inc., Groton, CT and* [2]*University of Houston, Houston, TX.*

Ocular drug delivery is impeded by numerous barriers the eye evolved to isolate and protect ocular tissues and preserve optimal visual function. For example, the blood-ocular barriers make delivery of therapeutic concentrations of systemically administered drugs more difficult. Local drug delivery (topical or peri-ocular) to ocular structures, at the front and back of the eye, are challenged by the various clearance and diffusional barriers such as lacrimation, mucosal barriers, cornea/sclera, choroidal, vitreous body, etc. Bypassing some of these barriers using direct injection or implants into the vitreous body carries safety, cost, and patient compliance concerns. Nanomaterials, because of their small size and potential to control surface properties and targeting, hold significant promise for improving transport of therapeutic agents across many of these ocular barriers. Hydrogels, polymeric micelles, liposomes, dendrimers, and cyclodextrans are just a few of the nanomaterials currently being developed for improved ocular drug and gene delivery. However, the potential ocular toxicity of these nanomaterials remains to be determined. Moreover, the efficacy and safety associated with their long- term use is unknown. The goal of the proposed session is to bring together academic and industry researchers, as well as government regulators, to further define and understand the unique safety issues confronting those that are developing and/or using nanoparticles for improved ocular drug delivery. The session will start with a broad overview of nanotoxicology and strategies for assessing the safety of nanoscale materials. The second speaker will focus on the current use of nanomaterials for enhanced delivery of ocular therapeutics to the eye. The third speaker will describe nanoparticles for the treatment of different diseases, particularly those associated with the retina and retinal pigment epithelium. The fourth speaker will discuss the retinotoxicity of current and proposed nanomaterials. The final speaker will address the current US FDA framework and strategies for regulating drug products using nanomaterials for ocular drug delivery. Special emphasis will be placed on the following emerging and evolving concepts as they relate to nano-sized drug delivery platforms for the eye: 1) current and future use of therapeutic nanomaterials for drug and gene therapies, 2) examples of nanomaterial-enabled ocular drug delivery platforms and associated toxicity evaluations, 3) mechanisms of retinotoxicity of nanomaterials, and 4) current US FDA thinking on efficacy and safety of nano-sized ocular drug delivery platforms.

W 1030b Overview of Nanomaterial Toxicology and Strategies for Assessing the Safety of Nanoscale Materials

N. J. Walker. *NIEHS-NTP, Durham, NC.*

There has been considerable research examining the potential utility of nanoscale materials and nanostructures in commercial and biomedical applications. Nanoscale materials (nanomaterials, nanoparticles) are a broadly defined set of substances that have at least one critical dimension less than 100 nanometers. The same novel chemical and physical properties that make nanomaterials useful also make their interactions with biological systems difficult to predict and evaluate in traditional toxicity models. The diversity in composition, size, surface coatings, and physico-chemical properties even within classes of "similar" nanomaterials can make translation of findings from one nanomaterial to another problematic. On the other hand, testing of each nanomaterial individually and defining what change in a physicochemical property constitutes creation of a "new" material, remains uncertain. Over the past years there has been considerable effort looking at developing guidance for assessing safety of nanomaterials. Some of the key considerations include; effective characterization of physicochemical properties of nanomaterials not only in the bulk phase but also within the toxicological test system; use of *in vitro* biological responses and shorter term *in vivo* studies of panels of nanomaterials to develop structure activity relationships for predicting hazard and prioritizing further targeted in-depth testing; and development of best practices to reduce potential interference of a nanomaterial with the test systems. This presentation will introduce the types and uses of nanoscale materials, current concepts in nanotoxicology, and the complexities and strategies of "nano" issues that must be addressed to assess the safety of these materials for biomedical applications for the eye and other tissues.

W 1031 Nanomaterials and Drug Delivery to the Eye

G. A. Lutty. *Johns Hopkins University, Baltimore, MD.* Sponsor: C. Somps.

The eye is a unique "closed," fluid filled compartment, making it easily amenable to intraocular drug delivery for many diseases. This presentation will focus on the current use of nanomaterials for enhanced delivery of ocular therapeutics to the eye. It will describe recent advances with bioerodable nanodevices and dendrimers as drug delivery nanomaterials and the methods of delivery (subconjunctivally, intravitreal, subretinal, or intravenously). The therapeutic targets are pathologies of the cornea or improvement in corneal transplantation, retina (ischemic retinopathies), and choroid (age-related macular degeneration or AMD). Our first efforts employed magnetic nanoparticles because we could easily attach DNA or peptides and they were easily purified. These were nontoxic, but had poor penetrance into retina and choroid when delivered intravitreally due to their large size (~100 nm). Pan et al. (*J. Control Release* 201:32-40, 2015) demonstrated that bioerodable nanoparticles (NPs) can be used to deliver steroids when given topically to reduce corneal graft rejection. Chitosan has been used to prolong drug life on the cornea, but is toxic when delivered intravitreally. Bioerodables like PLGA and PLA also have been evaluated by intravitreal injection and found to be nontoxic. However, the payload delivered to retina is limited, and the NPs do not penetrate retina. Dendrimers have great potential because they are nontoxic and can target activated microglia/macrophages, which are key cellular elements in many ischemic and inflammatory diseases of the eye (Kambhampati et al., Invest. Ophthalmol Vis Sci 56: 4413-4414, 2015). Their small size (~10 nm) and neutral charge allow them to pass through the fenestrations in the choriocapillaris endothelial cells so that they reach the choroid when administered intravenously. They penetrate the retina when given intravitreally and, when given intravenously in ischemia/reperfusion injury or during diabetic retinopathy, pass through a blood retinal barrier that has become permeable. Only dendrimers can be given intravenously and they target activated microglia/macrophages without a targeting ligand. In conclusion, bioerodable polymers and dendrimers are nontoxic, and can deliver drugs to the cornea and retina after intravitreal injection.

1032 Lack of Toxicity Associated with Nanoparticle-Mediated Gene Therapy for Ocular Diseases

M. I. Naash. *Department of Biomedical Engineering, University of Houston, Houston, TX*. Sponsor: C. Somps.

Mutations in genes expressed in the photoreceptors and retinal pigment epithelium (RPE) cause a wide range of blinding diseases including Leber's congenital amaurosis (LCA), Stargardt's macular dystrophy, retinitis pigmentosa (RP), and a variety of other forms of macular dystrophy. Since many of these diseases result from loss-of-function mutations, we have been exploring non-viral gene replacement therapy with compacted DNA nanoparticles (NPs). These NPs are composed of polyethylene-glycol conjugated polylysine and DNA, and for a typical expression plasmid have a minimum diameter of 9 nm. We conducted extensive studies demonstrating that they are well-tolerated and safe for use in the eye and can mediate long-term, (often for the life of the animal) phenotypic improvements in several mouse models of retinal diseases: even those associated with large genes. In spite of these positive results, clinical relevance requires further improvements in levels/distribution of expression and more importantly repetitive dosing must be feasible without toxicity. Our studies demonstrate that single or repetitive dosing of NPs carrying different vectors did not induce inflammation, whereas an early, transient inflammatory response was induced by the subretinal injection procedure. Also, there were no functional consequences of repetitive NP dosing, as assessed by ERGs, and no perturbation of endogenous RPE and photoreceptor gene expression as monitored by RPE65 and RDS mRNA levels, respectively. These findings extend prior benign NP toxicology results in the lung and brain, and indicate that repetitive ocular doses of DNA NPs may be non-toxic. Thus, we conclude that compacted DNA NPs have the potential to become clinically relevant treatments for a variety of inherited ocular diseases.

1033 Nanomaterials and Retinal Toxicity

W. K. Boyes. *US EPA, Research Triangle Park, NC.*

The neuroretina should be considered as a potential site of nanomaterial toxicity. Engineered nanomaterials may reach the retina through three potential routes of exposure including; intra-vitreal injection of therapeutics; blood-borne delivery in the retinal vasculature and then crossing the blood-retinal barrier; and through the choroidal blood supply, crossing the Bruch's membrane and the retinal pigment epithelium (RPE). The blood-retinal barrier is functionally similar to the blood-brain barrier, normally restricting transport of larger sized materials, but particles in the lower nanomaterial size range can be expected to transit. The blood flow to the retinal choroid is, on a tissue mass basis, one of the highest in the body raising the potential for rapid delivery of nanomaterials to the RPE. *In vitro*, RPE cells rapidly uptake nano particles, transport and agglomerate them in the perinuclear cytoplasm. *In vivo* studies have shown that the eye can uptake nanomaterials and retain them longer than many other tissues after cessation of exposure. Toxicity from nanomaterials to the neural retina or the RPE would be expected to follow common mechanisms identified for other tissues including generation of reactive oxygen species, alteration of cellular redox status, altered intracellular signaling, and release of toxic metal ions from soluble metallic particles. The retina and other ocular tissues, however, have potential for additional phototoxic mechanisms not relevant in other neural tissues due to the concurrent exposure to light. Titanium dioxide (TiO2) nanoparticles can be efficient photo-catalysts generating superoxide and hydroxide radicals in aqueous media under UV wavelengths of exposure resulting in phototoxic damage to surrounding tissues. For potential drug development, fullerene and related materials, including the hydroxylated derivative, fullerol, which have been proposed for applications as drug carriers, show phototoxicity under both UVA and visible wavelengths. The potential for retinal toxicity including phototoxicity of nanomaterial-enabled ocular drug preparations may be important to consider as a component of drug development and safety evaluations.

1034 FDA Perspective on Safety Assessment Needs for Nanoparticles Used for Drug Delivery to the Eye

W. A. Chambers. *US FDA, Silver Spring, MD.*

Ocular products containing nanomaterials are being investigated for potential applications as therapeutics. While some of these nanomaterials are nanoscale versions of larger materials used in approved products, other nanomaterials are novel and have never been used in ocular drug products. It is widely accepted that there are unique features associated with nanotherapeutic products, and these unique features may lead to future challenges for the development, manufacturing, safety evaluation and review of these products. These challenges are both for the sponsor of nanotechnology-based products, as well as for regulatory agencies, who must regulate these products. The FDA must ensure the safety and efficacy of nanomaterial containing ocular products, yet, it must also promote innovation in order to provide the public with access to the best medical products. This presentation will outline the current framework and developing strategy for the regulation of ocular drug products using nanomaterials.

1035a Scientific Reproducibility: Does This Pose a Problem for 21st Century Toxicology?

J. C. Rowlands[1] and A. Boobis[2]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Imperial College, London, United Kingdom.*

The safe use of chemicals relies upon the identification of exposure levels below which there is reasonable certainty of no harm. Traditionally, this has been achieved using studies in experimental animals prospectively and/or epidemiological surveillance prospectively or retrospectively. However, rapid advances in the development of mechanism-based biomarkers of disease, predictive *in vitro* assays and *in silico*-based computational modeling offer the prospect of more efficient, accurate, and relevant assessments. The application of these advances to human clinical and observational studies has the potential to provide investigators with a fuller understanding of human effects from exposure to environmental chemicals and responses to therapeutic agents. However, an increasing number of biomedical journals and funding agencies are raising significant concerns that much of what is published in the biomedical literature cannot be reproduced. Given that the published literature provides much of the basis for the fundamental understanding of a toxicants mechanism(s) of action, this lack of reproducibility raises important challenges for moving toxicology from an animal-based system to a system of mechanism of action-based toxicity predictions. Further, the impact on outcomes and confidence can be significant for human clinical and observational studies that rely upon biomarkers of human diseases. The workshop will comprise a series of highly focused presentations representing differing points of views of the extent, potential impacts, and solutions for the lack of reproducibility of published biomedical, epidemiological, and toxicological research. Each speaker will provide their views regarding the extent of the problem and, more importantly, approaches that are available to mitigate the problems.

1035b Reproducibility and Reliability of Biomedical Research: The Extent of the Problem

C. G. Begley. *TetraLogic Pharmaceuticals, Malvern, PA.*
Sponsor: J.C. Rowlands.

Biomedical and scientific advances are predicated on new knowledge that is robust and reliable and that serves as a solid foundation on which further advances can be built. Biomedical research is in the midst of a revolution with the generation of new data and scientific publications at a previously unprecedented rate. However, unfortunately, there is compelling evidence that the majority of these discoveries will not stand the test of time. This is a multifaceted, multi-stakeholder problem - no single party is solely responsible, and no single solution will suffice. This presentation will dissect 3 highly-cited publications, from leading laboratories, published in top-tier journals that illustrate the recurring lack of attention to rigor and reproducibility that currently pervades the biomedical literature. This problem would be largely addressed if experiments were performed blinded, repeated, included positive and negative controls, utilized validated reagents, showed all the results, and appropriately analyzed data.

1036 Providing Advice to Risk Managers Based on Imperfect and Potentially Incorrect Information

A. Boobis. *Imperial College, London, United Kingdom.*

Current approaches to the assessment of health and environmental risks frequently result in a variety of technical expressions of risks, based on consideration of endpoints, biological responses or other technical parameters. A lack of information, incomplete information, or incorrect information results in uncertainty in risk assessments. Therefore, the level of uncertainty depends on the quantity, quality, and relevance of data and on the reliability and relevance of models and inferences used to fill data gaps. This presentation will discuss the impact from relying on an imperfect database of biomedical research used to support risk assessments relied upon for decisions by risk managers.

W 1037 Evidence-Based Approaches for Enhancing the Reproducibility of Toxicological Studies

M. Stephens. *CAAT, John Hopkins University, Baltimore, MD.*

The reproducibility of scientific studies depends largely on the quality of their methodology and their reporting of findings. Studies with poor methodological quality (e.g., non-random allocation of animals to experimental and control groups) are subject to high systematic or random error, leading to spurious variability in outcomes. Such studies are hard to reproduce. Similarly, studies with poor reporting quality (e.g., no mention of housing and husbandry conditions) are also hard to reproduce (in the absence of contacting the authors). Evidence-based approaches, pioneered in clinical medicine (Evidence-Based Medicine) and currently being translated to toxicology (Evidence-Based Toxicology), provide tools and concepts that can be used to enhance the methodological and reporting quality - and thus the reproducibility - of toxicological studies. Systematic reviews, for example, include an assessment of the methodological quality of included studies. Such approaches can also used prospectively as guidance for enhancing the methodological quality of new studies. Guidance on reporting quality emerged initially for clinical studies, and more recently for preclinical studies on animals, which is applicable to animal-based toxicological studies. From the perspective of 21st century toxicology, evidence-based approaches provide guidance on the design and reporting – and hence the reproducibility - of studies using new methods.

W 1038 Interpreting Epidemiologic Studies: Quality Matters

J. LaKind. *LaKind Assc., Catonsville, MD.*

Epidemiologic studies evaluating associations between biomarkers of exposure to short-lived chemicals and health endpoints in humans face special challenges. Thus it is critical to evaluate the quality of epidemiologic studies that measure biomarkers of chemicals with short physiologic half-lives. To address these challenges, the Biomonitoring, Environmental Epidemiology, and Short-Lived Chemicals (BEES-C) instrument was developed to provide systematic guidance for evaluating 14 different aspects of study quality divided into three broad categories: 1) biomarker selection and measurement, 2) strategy and execution of exposure assessment, and 3) general considerations of study design and reporting. Case studies will be presented on the use of the BEES-C instrument, which demonstrates that most of its elements were effective in comparing the quality of available studies, with reviews generally concordant and justifications consistent. The strengths and limitations of BEES-C will be discussed.

W 1039 Addressing Data Reproducibility at the US Environmental Protection Agency Office of Research and Development

R. N. Hines. *US EPA, Research Triangle Park, NC.*

The lack of data reproducibility is recognized as a multifaceted problem. Contributing factors include a failure to adequately employ statistical considerations during study design, bias in sample selection or subject recruitment, errors in developing data inclusion/exclusion criteria, and flawed statistical analysis. However, there also are factors associated with the evolution of modern science that exacerbate the problem, some of which will be presented as discussion points. Although the US Environmental Protection Agency, Office of Research and Development, already has a strong Quality Assurance Program, several initiatives are underway to help ensure data reproducibility. Examples that will be discussed are the Executive Order for open access by developing infrastructure to make the data underlying publications open to the public, adoption of systematic review processes, and the development of value-added study design guidance documents.

W 1040 The Cancer Risk Assessment for Ingested Hexavalent Chromium: Challenges and Controversies

D. Proctor[1] and A. Jarabek[2]. *[1]ToxStrategies, Inc, Mission Viejo, CA and [2]US EPA, Research Triangle Park, NC.*

In 2008, the National Toxicology Program (NTP) released the findings of its two-year drinking water bioassay of hexavalent chromium (Cr[VI]). NTP concluded that there was clear evidence of carcinogenicity based on increased incidence of small intestinal tumors in mice (at 20180 mg/L), and oral cavity tumors in rats (at 60180 mg/L). The tumor data for the mouse small intestine have been used in several regulatory risk assessments from 2008 to 2011, and a linear low-dose extrapolation has been consistently applied based on the conclusion that Cr(VI) acts by an unknown or mutagenic mode of action (MOA). However, recent research challenges that characterization, supporting the hypotheses that intestinal tumors in mice arose by a non-mutagenic MOA involving cytotoxicity with regenerative hyperplasia and genomic instability associated with epigenetic mechanisms. Such data may have implications for low-dose extrapolation approaches for cancer risk assessment, and inform whether linear or nonlinear assumptions are appropriate. Furthermore, Cr(VI) is reduced to Cr(III) (and thus detoxified) in gastric fluid, a kinetic process which may greatly impact Cr(VI) absorption in the small intestine. The relevance of the dose levels administered to rodents by NTP to human exposure levels has also been debated. The lowest administered drinking water concentration was 5,000 ppb Cr(VI). Nationwide data collected by US EPA under the third Unregulated Contaminant Monitoring Rule (UCMR 3) shows that while approximately 80% of samples are below 0.5 ppb (10,000 fold lower than the lowest NTP concentration), 3% of samples were =5 ppb and <0.1% were between 50100 ppb. Recognizing the importance of both low-dose extrapolation and inter-species differences in gastrointestinal toxicokinetics, physiologically based pharmacokinetic (PBPK) models have been developed to estimate target tissue dose and inform these issues. This symposium will present diverse perspectives, with supporting data, regarding approaches for PBPK modeling and analyses of mechanistic data supporting hypothesized MOAs for Cr(VI)-induced gastrointestinal tract tumors. Resulting implications for dose-response extrapolation will be presented for discussion. (Views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.)

W 1041 Toxicokinetic Modeling of Cr(VI) in the Gastrointestinal Tract for Rodent-to-Human Extrapolation

A. Sasso. *US EPA National Center for Environmental Assessment, Washington, DC.* Sponsor: D. Proctor.

Cr(VI) has been shown to be reduced by gastric fluids collected from mice, rats, and humans. Extracellular reduction of Cr(VI) within the gastric lumen is believed to play a role in the detoxification of Cr(VI) and quantification is important for estimating target tissue dose. Reduction appears to occur as a pH-dependent, second order reaction, with multiple reduction pathways. The development of gastric reduction models for assessing the impact of gastrointestinal tract physiology on Cr(VI) reduction will be discussed. Multiple different types of models and gastric assumptions will be outlined. Using mouse small intestinal hyperplasia as an example, the impact of model uncertainty on rodent-to-human extrapolation will be illustrated. It was found that efficiency of Cr(VI) reduction can be greatly impacted by stomach pH and physiology, which are different between rodents and humans. There are also differences in diet and drinking water intake patterns, as well as gastric responses to ingested food, that may impact stomach reduction. Alternative toxicokinetic methodologies produce estimates of human equivalent dose that are within an order of magnitude. Therefore, it is feasible to perform rodent-to-human extrapolation using a simplified kinetic model of the stomach lumen. [The views expressed in this talk are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency].

W 1042 Mechanisms for Cr(VI)-Induced Carcinogenicity: Perspectives from Past and Current Research

J. P. Wise. *University of Louisville, Louisville, KY.*

Hexavalent chromium [Cr(VI)] has long been recognized as genotoxic; however, evidence has been building over the past decade supporting mechanisms other than base substitution mutations. Cr(VI) is transported into cells by anion channels where reduction to Cr(III) occurs, resulting in reactive oxygen species, DNA binding, protein modifications, and DNA methylation. Most studies indicate Cr(VI) is weakly mutagenic, and it is unclear if the mutations observed are due to direct DNA damage or other mechanisms. Newer mechanisms are proposed based on chromium's ability to destabilize the genome. This presentation will consider some of these new perspectives including microsatellite instability, centrosome amplification, defective DNA repair and induction of structural and numerical chromosome defects. It is increasingly clear that Cr(VI)'s mechanism likely involves an interaction between epigenetic changes and genotoxic events, although insights into the epigenetic aspects lag behind the well-established genotoxic aspects. These interactions have

important implications for determining key events for risk assessment. To highlight these emerging connections, the presentation will use the clastogenic effects of Cr(VI) as a case study and highlight how epigenetic modifications may inhibit the repair of these lesions and alter the regulation of cell cycling and subcellular organelles such as centrosomes.

W 1043 Non-Mutagenic MOA for Cr(VI) Involving Intestinal Cytotoxicity and Regenerative Hyperplasia

C. Thompson. *ToxStrategies, Inc., Katy, TX.*

Some quantitative risk assessments for Cr(VI) have been based on tumor incidence in the small intestine of mice. However, mice in the NTP study also exhibited non-neoplastic intestinal lesions including villous blunting and crypt hyperplasia—effects that were characterized as secondary to mucosal injury. Several studies designed to address the MOA for intestinal tumors in mice have been conducted since the NTP bioassay was completed. Collectively, these studies support that ingested Cr(VI) that is not reduced to inert Cr(III) by gastric fluid is passed into the intestinal lumen where it can be absorbed by villous enterocytes (key event 1). At high Cr(VI) drinking water concentrations, Cr(VI) causes cytotoxicity in villous enterocytes (key event 2). To compensate for villus damage, the proliferative crypt compartment becomes hyperplastic (key event 3). Under chronic high Cr(VI) exposure, sustained hyperplasia leads to increased spontaneous mutations in crypt stem cells (key event 4), which can ultimately result in tumorigenesis. Multiple *in vivo* genotoxicity assays in the small intestine (and other tissues) are negative, and indicate an absence of direct genotoxicity in the crypt compartment. Together, pharmacokinetic, histopathologic and genotoxicity data support a non-mutagenic, non-linear, threshold-based MOA for Cr(VI)-induced tumors in the small intestine of mice. A similar MOA has been proposed for captan and folpet, which suggests that these three chemicals may converge on an adverse outcome pathway (AOP) predictive of small intestinal tumors. In addition to the MOA discussion, a dose-response analysis is described that characterizes cancer risk from oral exposures to Cr(VI) that is based on non-neoplastic precursor lesions observed in the NTP cancer bioassay.

W 1044 Systematic Analysis of Mechanistic Information for Cr(VI)

C. Gibbons. *US EPA National Center for Environmental Assessment, Washington, DC.*

Although the human cancer risk from oral exposures to Cr(VI) has been debated for many decades, sufficient data to develop a cancer dose-response assessment were not available until the 2008 NTP 2-year bioassay reported clear evidence of carcinogenic activity in the gastrointestinal tract in both sexes of mice and rats following drinking water exposures. Use of these data to develop a quantitative risk estimate may be informed by the evaluation of mechanistic events that may provide relevance and context for the empirical evidence observed from a variety of human and animal studies. The National Research Council has emphasized the need for consistent, transparent, and systematic approaches for the identification, evaluation, and integration of data for assessing hazards to human health. Mechanistic studies present a challenge because they are typically both numerous and diverse and involve a multitude of targets and toxicity pathways. This challenge is particularly true for the mechanistic database for Cr(VI), which contains over 800 studies. This presentation discusses the findings of a systematic review of the evidence for mechanistic events that may be implicated in the carcinogenicity of ingested Cr(VI), including considerations regarding biological plausibility and human relevance, and how these data may be used to inform conclusions on MOA and the identification of susceptible populations and lifestages. [The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of the US Environmental Protection Agency or other affiliations.]

W 1045 Dose-Response Analysis Options for Hexavalent Chromium-Induced Cancers

M. Deveau[1] and I.D. Moffat[2]. [1]*University of Ottawa, Ottawa, ON, Canada and* [2]*Health Canada, Ottawa, ON, Canada.*

A major step in the derivation of exposure limits for oral chromium exposures is the quantitative dose–response assessment. The application of linear vs. non-linear approaches in a chromium risk assessment is dependent on decisions taken on the weight of evidence for mutagenic vs. non-mutagenic modes of action for hexavalent chromium. This presentation will build upon discussions from the previous speakers by demonstrating how mode of action analysis and PBPK modeling results can be differentially applied, resulting in quantitative variances in recommended exposure limits. The presentation will illustrate the application using two recently-proposed dose–response assessments. The first assessment concluded that due to evidence of mutagenicity at high doses, linear low-dose extrapolation was the most appropriate dose–response approach, and calculated a drinking water exposure limit of 0.02 µg/L (based on small intestine adenomas and carcinomas in male mice, and lung cancer in humans occupationally exposed to high inhalation doses of chromium). Conversely, an exposure limit of 100 µg/L was calculated when a PBPK model was used to demonstrate depletion of hexavalent chromium at low-dose exposures of humans; the non-linear analysis was performed using small intestinal hyperplasia as the critical effect, as a precursor for tumors generated by a non-mutagenic mode of action. In conclusion, varying assumptions on the most relevant mode of action for the exposure of humans to hexavalent chromium in drinking water can lead to qualitative and quantitative differences in applied dose–response approaches.

W 1046a Transient Receptor Potential A1 (TRPA1) Cation Channels: Fluttering Hearts, Headaches and Hot Flashes—Can One "Environmental Sensor" Be the Cause of All the Pain?

M. S. Hazari[1] and D. J. Conklin[2]. [1]*US EPA, Research Triangle Park, NC and* [2]*University of Louisville, Louisville, KY.*

The role of transient receptor potential (TRP) cation channels, particularly TRPA1, in numerous toxicological pathways has garnered great interest over the last decade. These investigations have focused on the ability of TRPA1 to act as an environmental sensor for chemical irritants such as acrolein, formaldehyde, and chloramines, which are found in disinfectants, but also as a mediator of pain, inflammation, and acute physiological changes given it is a target of reactive oxygen species such as hydrogen peroxide and even endogenous agonists such as 4-hydroxy-nonenol. These features, as well as its presence on sensory neurons throughout the body, make TRPA1 an important component in the mode of toxicity of not only gaseous irritants, but also chemicals transmitted in water or by direct contact with the skin. Although it has become clear that TRPA1 contributes to the acute adverse health effects of exposure, new studies have now revealed that it is also involved in the development and progression of chronic diseases. This latter phenomenon is likely related to variations in the TRPA1 gene, which manifest at a young age, but also due to a lifetime of accrued epigenetic changes that essentially alter the responsiveness of an individual to a given exposure. Therefore, this session will highlight the newly understood multifaceted roles of TRPA1 in environmental sensing. The presentations will focus on a broad array of topics including how TRPA1 mediates acute cardiovascular effects after inhalation of air pollutants. Findings will show that inhalation of not only typical TRPA1-activating gaseous irritants, but also particulate matter, causes cardiac arrhythmogenesis as well as vascular responses through TRPA1- mediated mechanisms. Attendees will also hear about the role of TRPA1 in the masking effects of flavorants such as menthol and cinnamon in e-cigarettes, which has important implications in both studying the health effects of these alternative tobacco products and their potential regulation. In addition, chemical sensitivity to environmental irritants will be explored in relation to TRPA1 and trigeminal-vascular mechanisms, which are nasal sensory pathways that alter blood flow to the brain and are linked to episodic headaches. Finally, the broader implications of TRPA1 variability, lifestyle, and environment will be addressed from an epigenetic perspective in a study of identical twins to demonstrate links to susceptibility and chronic pain.

W 1046b TRP Ion Channels as Key Targets of Tobacco and Electronic Cigarette Irritants and Flavor Additives

S. E. Jordt. *Duke University, Durham, NC.*

The physiological effects and toxicities of flavor additives in tobacco products and electronic cigarettes remain poorly understood. Menthol is by far the most popular flavorant and may facilitate smoking initiation, potentiate the toxicological effects of smoking and reduce quitting success. Other added flavorants are known to cause irritation and inflammation. However, it is unclear if these effects occur at concentrations present in electronic cigarette vapor. Using plethysmography in a mouse model, we examined the effects of L-menthol, the menthol isomer added to cigarettes, on the respiratory sensory irritation response to smoke of Kentucky reference 2R4 cigarettes. We also studied L-menthol's effect on blood levels of the nicotine metabolite, cotinine, immediately after exposure to cigarette smoke. Cigarette smoke (10 mg/m3 or higher) caused an immediate and marked sen-

sory irritation response. This response was significantly suppressed by 19-60ppm L-menthol even at smoke concentrations as high as 300 mg/m3. Inhibition by L-menthol was abolished by treatment with AMG2850, a selective inhibitor of TRPM8, the neuronal cold/menthol receptor. Inclusion of menthol in the cigarette smoke resulted in roughly a 1.5-fold increase in plasma cotinine. Fluorescent calcium imaging in HEK293 cells revealed that dilutions of flavored electronic cigarette liquids potently activated the cloned irritant receptors, TRPV1 and TRPA1. Cinnamon-flavored liquids, some of which contain >10% cinnamaldehyde, a potent sensory irritant, strongly activated TRPA1 receptors, even at high dilution factors (>1:1000). Other flavors, including carvone- and methyl salicylate-containing solutions, activated both TRPV1 and TRPA1 receptors and minty flavor solutions activated the cold/menthol receptor, TRPM8, known to suppress respiratory irritation. These effects also occurred with nicotine-free flavored solutions. Ongoing studies examine whether e-cigarette vapors cause inflammation in the mouse airways, and whether these responses depend on specific TRP irritant receptors. Taken together, these data show that flavorants in tobacco and electronic cigarettes target TRP ion channels to control irritant responses and, in the case of menthol, increase critical markers of nicotine uptake. Thus, flavorants may modify smoking behavior and promote chronic irritation and inflammation in the airways.

W 1047 Examining the Role of TRPA1 in Air Pollution-Induced Cardiac Arrhythmias and Autonomic Imbalance

M. S. Hazari. *US EPA, Research Triangle Park, NC.*

Time-series studies show the risk of adverse cardiac events increases significantly in the hours to days after exposure to air pollution. Although activation of sensory neural arcs and autonomic imbalance have been proposed as an important mechanism of air pollution-induced cardiovascular dysfunction, little data exists on the specific pathways that mediate these sudden cardiovascular outcomes (e.g. arrhythmia). Neural sensory networks play a role in not only the immediate response to a pollutant, but also an autonomic shift that renders the host susceptible to adverse cardiovascular events in the hours after exposure. TRPA1 is a suitable candidate for initiating these mechanisms given it functions as an environmental sensor of several ubiquitous environmental chemicals and is localized to nociceptive nerve fibers that trigger autonomic reflex arcs and subsequent physiological changes. Using radiotelemetry and *ex vivo* cardiac reperfusion, we measured cardiac function in wild-type and TRPA1-knockout mice exposed to 3ppm acrolein. Our results show that a single inhalation exposure to acrolein causes TRPA1-dependent non-conducted p-wave arrhythmias, sinus node dysfunction, and a significant increase in heart rate variability (SDNN/RMSSD), suggesting disruption of the normal electrical signal and altered autonomic modulation, respectively. TRPA1 also mediated a 50% increase in left ventricular developed pressure (LVDP), a measure of cardiac mechanical function, 24 hours after exposure. Furthermore, our studies in rats show that diesel exhaust (150 ug/m3) exposure increases sensitivity to the pro-arrhythmogenic drug aconitine through TRPA1 and autonomic sympathetic modulation. Taken together, these data show that TRPA1 mediates cardiac arrhythmia during air pollution exposure and increases the risk of arrhythmogenesis after exposure. On the other hand, it may also cause disruption of the autonomic control of the cardiovascular system and compensatory mechanical changes, both of which are latent alterations that make the host susceptible to triggered adverse events. In conclusion, although the role of TRPA1 appears to be clear, these data point to the complexity and dynamic nature of these neurophysiological pathways that transduce inhalation of air pollution into cardiovascular effects. This abstract does not reflect US EPA policy

W 1048 From Tobacco Smoke to Gas Attacks–How TRPA1 Modulates Cardiopulmonary Toxicity of Acrolein

D. J. Conklin. *University of Louisville, Louisville, KY.*

Acrolein is encountered daily from very low environmental levels up to higher levels as in tobacco smoke and in structural and wildfires. Given the high sensitivity of the cardiopulmonary system to volatile toxics such as acrolein and the potential role of TRPA1 channels, we tested whether acrolein-induced effects across three orders of magnitude are modulated by TRPA1 in mice. Low-level acrolein (1 ppm, 6hours/day for 4 days) induces a reversible, dose- and time-dependent suppression in circulating endothelium progenitor cells (EPCs) and a corresponding increase in bone marrow EPCs. Coincident with EPC changes, we observed vascular resistance to exogenous vascular endothelial growth factor (VEGF) signaling. These effects were absent in similarly exposed TRPA1-knockout mice implicating TRPA1 channel dependence in these systemic effects. In contrast, exposure to high-level (>200 ppm, 30 min) acrolein induced pulmonary epithelium injury, massive inflammation, systemic depletion of lymphocytes and lethality in TRPA1-null mice in a gene-dose and sex-dependent manner compared with wild type mice (i.e. lack of TRPA1 worsened the response). Treatment of mice after a high-level acrolein exposure with a TRPA1 antagonist was ineffective in rescue from morbidity and mortality. Thus, these data suggest that TRPA1 mediates systemic effects of low-level acrolein exposure, yet, it also appears to provide measurable protection against high-level acrolein exposure. Prophylactic treatment with TRPA1 antagonists, however, may lead to either protection or injury dependent on acrolein level, and thus, must be exercised with caution. There continues to be a need to better understand the mechanisms by which acrolein induces cardiopulmonary injury to develop more targeted treatments.

W 1049 TRPA1 and Meningeal Vasodilatation Due to Environmental Irritants–Mechanisms of Migraine

J. Hurley. *Indiana University, Indianapolis, IN.* Sponsor: M.S. Hazari.

Headache is one of the most common complaints of exposure to environmental irritants and air pollutants. The pain from headaches occurs due to activation of the trigeminovascular system, including the trigeminal neurons and meningeal blood vessels they innervate. Trigeminal sensory neurons express excitatory TRPA1 receptors targeted by environmental irritants such as formaldehyde and acrolein. Stimulation of these neurons releases CGRP, a potent vasodilator that is associated with the pain of migraine. As such, migraine is an episodic disorder which in some patients worsens to a chronic condition with increased emergency room visits and patients frequently citing chemicals or odors as triggers. Thus, although headaches have been widely reported and shown to be induced by inhalation of environmental agents, it is unclear how these agents produce their effects. We show here that activation of TRPA1 receptors is the mechanistic link between environmental irritants and stimulation of the trigeminovascular pathway, and that environmental irritants (TRPA1 agonists) acutely increase meningeal blood flow following intranasal administration and stimulate CGRP release from trigeminal neurons leading to headaches. Meningeal blood flow was measured in rats using a laser Doppler flowmeter while solutions of the TRPA1 agonists acrolein or mustard oil were administered to the nasal mucosa. A two-fold increase in peak blood flow was observed with both agonists which was blocked by a CGRP antagonist applied to the dura or pretreatment of the nasal mucosa with a TRPA1 antagonist. We also confirmed the release of CGRP from rat trigeminal neurons in culture in a TRPA1-dependent manner upon stimulation with mustard oil. Furthermore, chemical exposure has also been linked to Multiple Chemical Sensitivity, an acquired disorder, in which headache, respiratory and skin problems are common. Therefore, we examined whether subacute chronic environmental irritant exposure sensitizes the trigeminovascular system. We showed that inhalation exposure to acrolein sensitizes meningeal blood flow responses to subsequent nasal administration of TRPA1 agonists compared to room air. Although the mechanism of sensitization remains to be determined these results suggest an important role for TRPA1 receptors in enhanced headache susceptibility after chemical exposure.

W 1050 Epigenetic Changes in the TRPA1 Promoter Show Pain Sensitivity Is Affected by Lifestyle and Environment

S. McMahon. *London Pain Consortium, King's College, London, London, United Kingdom.* Sponsor: M.S. Hazari.

Chronic pain is a global public health problem, but the underlying molecular mechanisms are not fully understood. Aside from the innate genetic predisposition, an individual's responsiveness to painful stimuli (e.g. heat) is likely altered over a lifetime by not only lifestyle but environment as well. This presentation will describe the results of a study in which we explored genome-wide DNA methylation profiles in 100 individuals, consisting of 50 identical twins discordant for heat pain sensitivity (heat pain tolerance > 2 degrees C between twins assessed using quantitative sensor testing), and then 50 further unrelated individuals to demonstrate the role of TRPA1, pain sensation and the role of the epigenome in modulating it's response over a lifetime. Using whole-blood, epigenetic changes were assessed to identify regions associated with high or low pain sensitivity (pain DNA Methylation Regions - DMRs). We show here that nine meta-analysis pain DMRs have robust evidence for association with the strongest signal in the promoter of the TRPA1 gene. The TRPA1 promoter pain DMR in our data was hypermethylated in individuals with lower pain thresholds, and promoter DNA methylation can downregulate gene expression. To assess whether promoter DNA methylation differences in TRPA1 reflected functional consequences in pain, we obtained gene expression data from skin biopsies in unselected normal female twins. We observed a modest nominally

significant increase in TRPA1 gene expression levels in individuals with higher pain thresholds, consistent with the hypermethylated pain DMR result. Other regions among the top-ranked 100 were for TRPV1 and TRPV3. Furthermore, several pain DMRs showed longitudinal stability consistent with susceptibility effects, had similar methylation levels in the brain and altered expression in the skin. Finally, this presentation will discuss epigenetic changes in both novel and established pain candidate genes as well as molecular insights into pain that may generalize to other complex traits.

1051a The Toxicological Implications of the Gulf Oil Spill: Research Accomplishments and Research Needs

B. Goldstein[1] and M. Lichtveld[2]. [1]*University of Pittsburgh, Pittsburgh, PA and* [2]*Tulane University, New Orleans, LA.*

This session will focus on existing toxicological research concerning the impact of the Gulf Oil Spill on human health, discuss the many remaining uncertainties requiring toxicological research, and review the availability to toxicological scientists of the more than $1 billion that has or will be spent in different programs on research related to preventing and to understanding the potential impact of future oil spills affecting American coastal waters and communities. More than five years ago the Deepwater Horizon explosion released millions of barrels of oil into the Gulf of Mexico over a five-month period. The chemical and physical characteristics of this crude oil changed over time because of its interaction with sea, shore and sun. Much of it was burned at sea, releasing combustion products. An unprecedented amount of a chemical dispersant sprayed on the oil spill added to the potential toxicological implications. The potential impact on seafood safety also has been a significant issue, with concern both about health effects to those ingesting seafood as well as the economic and social implications to Gulf communities dependent on gathering seafood. After the spill, a Society of Toxicology Working Group developed a two-page description of the toxicological implications aimed broadly at the public and at decision makers: www.toxicology.org/pubs/docs/pr/toxtopics/deepwater_oil_spill.pdf. This serves as a baseline for the proposed seminar that will be co-chaired by Maureen Lichtveld, the chair of the Department of Global Environmental Health Sciences at Tulane University School of Public Health and Tropical Medicine, who has been actively involved in the oil spill response, and Bernard Goldstein of the University of Pittsburgh, who has reviewed the subject with Dr. Lichtveld (New Engl J Med 364:1334–1348, 2011) and has been significantly involved in three of the programs funding oil spill activities. Dr. Goldstein was also involved in the SOT Communications Strategy that led to the SOT Working Group document. The opening presentation will be delivered by Linda Birnbaum, director of the NIEHS, who will provide an overview of the NIEHS response that has led to funding for the large majority of oil spill toxicological research and for future research needs. This will be followed by scientists directly involved in research related to the oil spill. Cornelius Elferink of the University of Texas Medical Branch will present studies related to petrogenic PAH contamination in Gulf seafood and its implications to local seafood consumers, including use of a PAH assay dependent on aryl hydrocarbon receptor responsiveness. Jeffrey Wickliffe will present information from the NIEHS consortia (Tulane, Texas Medical Branch, University of Florida and Louisiana State University) relevant to assessing the risks of seafood contamination. Dr. Lichtveld will discuss the utilization of toxicological research in epidemiological studies evaluating the impact of the oil spill and other Gulf area stressors on the health of vulnerable populations. Last, Dr. Goldstein will build upon the previous presentations, discuss the extent to which toxicological research has provided answers to the questions about potential human health impacts raised by the SOT Working Group, and describe the many active research initiatives providing funding for oil spill research and critique the extent to which toxicological research has been included.

1051b Gulf Oil Spill Response: Health Research, Community-Academic Partnerships, Lessons Learned, and Future Preparedness

L. S. Birnbaum. *National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

Relatively few of the approximately 40 major oil spills in the last 50 years have examined the health impacts on local communities and exposed populations. Typically, these studies lack measures of exposure and look only at acute health impacts of workers. This is also true of most disasters, where longitudinal health studies and associated exposure information are not available. In this regard, NIH/NIEHS intramural and extramural funded investigators, in partnership with workers and numerous community groups, have engaged in an array of research efforts to better understand the health impacts of the Gulf Oil Spill including epidemiologic investigations of over 32,000 clean-up workers (GuLF Study) and other at-risk community populations, large-scale exposure reconstructions, toxicology research into seafood contamination and PAHs, and development of education and training efforts to better protect those involved. While results continue to be gathered and evaluated, early findings underscore the need for further investigation to understand the long-term effects on established cohorts, risk factors and biomarkers (including genetic & epigenetic, susceptibility and disease, and the complex stressors and interactions) that contribute to community and individual resiliency or vulnerability. The evolution from design through implementation of these and other research efforts, in combination with novel community-academic partnerships, affords an opportunity to share key findings to further understanding of the health impacts of the Gulf Oil Spill, as well as chart new strategies to unravel the complex interplay and interdependence between human health and the environment and to support the development of healthy and resilient communities. Using these new platforms and "lessons learned" we can begin to translate "research to practice" to promote environmental health literacy, improve environmental and human health baselines, and further understanding of the toxicologic effects and risk factors associated with adverse outcomes. Lastly, continued development of multidisciplinary research and collaborations will help to improve the health and wellbeing of Gulf Coast communities and better prepare us to address the challenges of acquiring timely health data to improve preparedness and response for future disasters.

1052 A Toxicological Assessment of Petrogenic PAH Contamination in Gulf Seafood Following the Deepwater Horizon Oil Disaster

C. J. Elferink. *University of Texas Medical Branch, Galveston, TX.*

Utilizing a community-based participatory research approach involving Gulf coast subsistence fishing communities, the Gulf Coast Health Alliance: health Risks related to the Macondo Spill (GC-HARMS) consortium conducted a four-year longitudinal study of PAH contamination in US Gulf of Mexico seafood following the 2010 Deepwater Horizon disaster. Given the dearth of toxicology studies on petrogenic PAHs, we employed the EPA-approved Chemical Activated Luciferase gene eXpression (CALUX®) bioassay—based on Aryl Hydrocarbon Receptor (AhR) responsiveness to polycyclic aromatic hydrocarbons. Use of the CALUX assay is predicated on the assumption that petrogenic PAHs will elicit an AhR-mediated response in the assay. Our CALUX experiments tested PAH extracts from 432 total samples collected from either regions affected by the Deepwater Horizon oil, or control regions not directly oiled during the spill. Benzo[a]pyrene was used as the reference standard in comparison to the seafood extracts, to calculate BaPTEQ/g of seafood sample (wt/wt). In keeping with the principles of community based participatory research, we generated conservative seafood consumption guidelines for our community partners recognizing that seafood consumption by these communities greatly exceeds the national average. Supported by U19ES020676.

1053 Human Health Risk Assessments Regarding Consumption of Fish and Shellfish From the Northern Gulf of Mexico Following the Deepwater Horizon Accident: Results Across Three Academic Research Consortia

J. Wickliffe. *Tulane University, New Orleans, LA.*

During and following the Deepwater Horizon accident in April 2010, the public from producers to consumers was concerned about the safety of consuming fish and shellfish from the northern Gulf of Mexico. Perceptions among Gulf communities as well as consumers across the United States and beyond generally considered that contamination of seafood along the Gulf coast with chemicals from the crude oil spill was a foregone conclusion. A number of state programs as well as academic-community research teams began a number of independent fish and shellfish testing programs looking at the levels of chemicals of health concern known to be in crude oil. Three academic consortia funded through an NIEHS initiative to address health concerns regarding chemicals emanating from or used on the oil spill specifically sampled distinctly different sources of seafood (i.e. fish and shellfish) for chemical contaminants using an almost unprecedented, harmonized analytical approach. Polycyclic aromatic hydrocarbons (PAHs) were considered the chemicals of highest concern in crude oil that can adversely impact human health following long-term consumption. Health risk assessments were conducted independently by the three research consortia using project-specific PAH levels and population-specific parameters such as consumption rates and body weights. These assessments revealed that across these consortia, health risks regarding cancer as a

major long-term health endpoint were not elevated and not considered unacceptable even when considering long-term exposure. The approaches and results of these comprehensive health risk assessments regarding PAHs in sampled Gulf fish and shellfish will be discussed in detail.

1054 Linking Bench to Trench: Embedding Toxicological Science in Environmental Epidemiologic Studies to Unravel Complex Health Threats in Gulf Coast Reproductive-Age Women and Infants

M. Lichtveld. *Tulane University, New Orleans, LA.*

Communities living on the US Gulf Coast face a triple health threat: persistent risks associated with exposures to hazardous substances, a historic burden of health disparities, and the impact on their health of living in a disaster-prone area. From infant mortality to key chronic disease conditions, Louisiana's vulnerable populations, especially pregnant women and children, rank at the bottom of most health indicators. In the aftermath of the Gulf of Mexico (GOM) oil spill, Louisiana's coastal communities identified three key concerns: What will happen to our babies?, Is the seafood safe to eat?, and Is the air safe to breathe? The Center for Gulf Coast Environmental Health Research, Leadership, and Strategic Initiatives deploys transdisciplinary research approaches to address each of those questions engaging community partners from design to dissemination. The Gulf Resilience on Women's Health (GROWH) research consortium, embedded in the center, is a community-academic partnership unraveling the potential impact of the GOM oil spill on reproductive outcomes, infant neurodevelopment, and environmental health risk. Exposures to a complex set of chemical and non-chemical stressors in women of reproductive age and their infants were measured through a portfolio of biomarker assessments including epigenetic markers and psychosocial instruments. Early findings show environmental media contamination, a relationship between infant telomere length and blood lead levels, and cultural influences in risk assessment and management. The impact on birth outcomes measured by both adverse and protective biomarkers potentially associated with seafood consumption, will be presented. Integral to our research is community-engaged translation, dissemination and outreach. This presentation will also include examples of how research findings are translated into environmental health literacy interventions.

1055 Opportunities and Needs for Toxicological Research Related to the Gulf Oil Spill

B. Goldstein. *University of Pittsburgh, Pittsburgh, PA.*

Dr. Goldstein will focus on research needs and opportunities. He will build upon the previous presentations in this symposium and will discuss the extent to which the available scientific information about the potential for human health effects has moved ahead since the SOT review of this subject in Tox Topics (www.toxicology.org/pubs/docs/pr/toxtopics/deepwater_oil_spill.pdf) and since Dr. Goldstein and his coauthors, including Dr Lichtveld, published an invited review of the Gulf Oil Spill (*New Engl J Med* 364:1334-48, 2011). While significant progress has been made in understanding the psychosocial and community impacts of the spill, particularly on vulnerable populations, and there are some studies of the impact of weathered crude oil and dispersant on aquatic life, much more needs to be known to be responsive to public concerns about the potential for adverse human health effects. Important gaps remain in the understanding of the toxicological effects of weathered petroleum and of dispersants, alone and in combination, as well as their combustion products when burned at sea. Gaps are particularly evident in the ability to respond to public questions about reproductive and developmental toxicity, and about seafood safety. Many opportunities exist for the funding of toxicological research. The Gulf of Mexico Research Initiative (GOMRI), an independent research organization funded by BP, is in the process of disbursing up to $500 million over ten years. Although human health is one of its five themes, it has thus far received and funded only a relatively small amount of toxicological research. More recently, the National Academy of Science has received $500 million to fund research over a 30 year period related in part to protecting human health from oil spills in the coastal United States. With continued likelihood of additional oil spills it is important that toxicologists take advantage of these opportunities to demonstrate the value of toxicological science to the protection of public health and the environment.

1056 Time-Course Interactions Between Cell Proliferation and DNA Sequence Variants in a Mouse Model of Latent Carcinogenicity

C. E. Wood, W. Ward, B. Chorley, G. Carswell, A. Fisher, A. DeAngelo and S. D. Hester. *US EPA, Research Triangle Park, NC.*

A fundamental principle of non-mutagenic chemical carcinogenesis is that increased cell proliferation enhances spontaneous DNA damage. Over time, this damage drives mutations in oncogenic genes that ultimately lead to cancer. This concept is a central part of cancer mode of action evaluation, although few studies have quantitatively tested this idea. Here, we used a mouse model of latent carcinogenicity to examine interactions between cell proliferation and DNA sequence variants over time. The treatment for this study was dichloroacetic acid (DCA), an oxidative metabolic agent that induces liver tumors in mice in the absence of direct short-term mutagenic effects. Male B6C3F1 mice 4 weeks of age were treated with deionized water (dH20; control); 3.5 g/l DCA continuously; or 3.5 g/l DCA for 10 weeks followed by control dH20. We predicted that variants would increase with age and that DCA treatment would amplify this effect. Sequence variants in liver DNA were evaluated in 1348 target loci across 65 key cancer-related genes at 14, 30, 56, and 82 weeks of age using the Illumina TruSeq amplicon platform for targeted resequencing (n=4/group). Incidence of hepatocellular adenoma or carcinoma was higher in the continuous DCA (44/44, 100%) and prior DCA (34/55, 62%) groups compared to controls (19/52, 37%) ($P<0.01$ for both). Liver cell proliferation (labeling index) was modestly higher after 10 weeks of DCA treatment ($P=0.01$) but not at any subsequent time points, decreasing with age in all groups. At the 82-week time point, total variant counts were higher in continuous DCA (n=200) and prior DCA (n=174) groups compared to controls (n=99) ($P<0.001$ for both). However, absolute variant counts did not increase with age in any group. These findings support the idea that early-life epigenetic changes can lead to later-life genetic effects related to cancer. (This abstract does not reflect US EPA policy.)

1057 Metabolic Enzyme Activity Is Not Necessary for CAR-Mediated Liver Tumor Formation: A Case Study with Two Agrochemicals

M. J. LeBaron[2], J. LaRocca[1], M. Schisler[2], L. Kan[2] and R. J. Rasoulpour[1]. [1]*Dow AgroSciences, Indianapolis, IN and* [2]*The Dow Chemical Company, Midland, MI.*

There are several well-characterized modes of action (MoAs) for hepatocarcinogenesis, and the analysis of robust mechanistic data can provide a reasonable basis for extrapolation of a rodent MoA to human relevance. Nuclear-receptor mediated increase in cell proliferation is a well-known MoA for rodent hepatocarcinogenesis, with phenobarbital (PB) as the standard example. For constitutive androstane receptor (CAR)-mediated rodent hepatocarcinogenesis, the first key event is activation of the CAR signaling pathway, which is often measured via the empirically observable biomarker of Cyp2b10 induction (transcript, protein, and/or enzyme activity of 7-pentoxyresorufin-O-dealkylase (PROD)). The agrochemicals pronamide and nitrapyrin induce hepatocellular tumors in mice, and both molecules have a robust package of data demonstrating that CAR induction is the MoA. The exception for both molecules, however, is there is no associated change in PROD activity. Given the paradoxical finding of increases in Cyp2b10 for both molecules with no associated change in PROD, *in vitro* experiments were conducted with phenobarbital (PB)-induced liver microsomes to investigate the role for suicide inhibition. Microsomes were treated with PB (negative control), curcumin (positive control), nitrapyrin, or pronamide. In this system, nitrapyrin and pronamide inhibited PROD activity in a dose-related manner and up to 96% and 56% at 500 μM, respectively. These results indicate that the two agrochemicals and/or their metabolites irreversibly inhibit Cyp2b10-mediated PROD activity, which elucidates the apparent inconsistency between protein levels and enzyme activity of nitrapyrin- or pronamide-treated livers. Hence, the lack of PROD activity does not indicate a lack of CAR-mediated activity; in fact, the key event of CAR-activation is supported by gene and protein expression of Cyp2b10 for both molecules. These data support that endpoints other than enzyme activity (transcript, protein measurements) may be more appropriate and relevant for investigating this MoA.

1058 Mode of Action (MOA) of Triclosan Induced Mouse Liver Tumors

Z. Wang, X. Li and J. E. Klaunig. *Indiana University, Bloomington, IN.*

Triclosan (2,4,4'-trichloro-2'-hydroxy-diphenyl ether, TCS) is an antibacterial and antifungal agent used in some consumer products. Chronic dietary exposure to TCS produced an increase in liver tumors in CD-1 mice. TCS was not genotoxic in *in vitro* and *in vivo* bioassays. The MOA for TCS liver tumors has been attributed through activation of peroxisome proliferator activated receptor α (PPARα) (Rodricks et al., 2010). In contrast, (Yueh et al., 2014) reported that TCS activates the constitutive androstane receptor (CAR) while having no effect on PPARα. One reason for the differences in the results of these studies may be related to the mouse strains used. To resolve these discrepancies the current study was performed. Male CD-1 and C57BL/6J mice (5/group) were treated via diet with TCS at 0, 10, 100 and 200 mg/kg/day for 14 days. Additional mice were treated with phenobarbital (PB) or diethylhexyl phthalate (DEHP) to serve as controls for CAR or PPARα activity, respectively. TCS induced a dose-dependent increase in relative liver weight and centrilobular hypertrophy in both strains. Hepatocyte DNA synthesis was also increased in a dose related pattern. In the current study TCS induced a significant increase in CAR (Cyp2b10, Cyp3a11) and PPARα (Cyp4a10) responsive genes in both CD-1 and C57BL/6J mice. Correspondingly, the enzyme activity for CAR (7-pentoxyresorufin-O-dealkylase (PROD)) and PPARα (peroxisomal Acyl-CoA oxidase (ACO)) were also significantly increased in a dose-responsive manner. AhR expression (Cyp1a1, Cyp1a2) and activity (Ethoxyresorufin-O-deethylase (EROD)) was not increased by TCS treatment. In both strains, focal necrosis with inflammatory infiltration were observed in the TCS high dose groups. The present results resolve the difference in the previous reports on the mechanism of TCS induced mouse liver tumors. Both CAR and PPARα activation appear to be involved in the TCS induced mouse liver tumor MOA. The previously reported differences in the receptor activation is not strain related and may be related to experimental differences.

1059 Histone Modification Changes Reveal Target Organ Carcinogenicity of the Non-Genotoxic Liver Carcinogen Methapyrilene in Fischer 344 Rats

S. Shpyleva, I. Kindrat, F. A. Beland, T. Chen and I. P. Pogribny. *National Center for Toxicological Research, Jefferson, AR.*

Continuous exposure to an increasing number of certain natural and man-made chemicals is a major risk factor for cancer. Therefore, evaluating the carcinogenic risk of chemicals remains important. Currently, substantial progress has been made in identification of genotoxic carcinogens. In contrast, predicting a carcinogenic potential of non-genotoxic compounds is a challenge due to many different modes of action that may lead to tumorigenesis; however, accumulating evidence suggests that some of the earliest events of tumorigenesis involve perturbations of the cellular epigenome. In the present study, we investigated the effect of the non-genotoxic liver carcinogen methapyrilene, the genotoxic kidney carcinogen aristolochic acid, and the non-genotoxic non-carcinogen usnic acid on epigenomic alterations in the liver and kidneys of Fischer 344 (F344) rats. Male F344 rats were treated with methapyrilene (40 mg/kg body weight (bw)/day), usnic acid (60 mg/kg bw/day), or aristolochic acid (1 mg/kg bw/day), doses exhibiting little or no hepatotoxicity, by gavage for 6 weeks. Treatment of F344 rats with methapyrilene caused a marked decrease in the trimethylation of histone H3 lysine 4 (H3K4me3) and H4 lysine 20 (H4K20me3), and acetylation of histone H3 lysine 9 (H3K9ac) and H3 lysine 56 (H3K56ac) in the livers. Exposure to usnic acid resulted in a minor reduction of H3K4me3 and H3K9ac only. No histone modification changes were found in the livers of rats treated with aristolochic acid. Also, none of the chemicals caused histone modifications in the kidneys. The methapyrilene-induced hepatic histone modifications were accompanied by a reduced expression of chromatin-modifying genes responsible for the modifications, including histone methyltransferases Kmt5c and Kmt2a, histone demethylase Kdm5b, and histone acethyltransferase Ep300. The results indicate that organ specific carcinogen-induced histone modifications changes may have significance in the carcinogenic process and in carcinogen hazard identification.

1060 Interlaboratory Transferability of a Multiplexed Flow Cytometric Assay That Provides Information on Genotoxic Mode of Action

S. Bryce[1], D. Bernaki[1], J. Bemis[1], S. Dertinger[1], R. Spellman[4], M. Engel[4], M. Schuler[4], A. Zeller[6], A. Sutter[5], S. Wilde[5], U. Hemmann[3], M. Ruppert[3], B. Meyerhoefer[3], E. Lorge[2] and P. Heikkinen[7]. *[1]Litron Labs, Rochester, NY; [2]Servier Group, Gidy, France; [3]Drug Disposition, Safety and Animal Research, Sanofi-Aventis, Frankfurt, Germany; [4]Genetic Toxicology, Pfizer, Groton, CT; [5]Investigational Toxicology, Bayer HealthCare AG, Berlin, Germany; [6]Pharmaceutical Research and Early Development, F. Hoffman-La Roche, Basel, Swaziland and [7]Research and Development, Orion Corporation, Esspo, Finland.* Sponsor: S. Dertinger.

The objective of the current work was to assess the transferability of a novel high throughput assay consisting of biomarkers associated with DNA damage response pathways multiplexed into a single add-and-read-type assay that is analyzed via flow cytometry. As part of the transferability exercise, Litron tested 69 compounds in human TK6 cells and those data served as a training set for a mode of action prediction model. Several external collaborating labs studied a pre-defined subset of compounds from the training set over a range of concentrations in 96 well plates. At 4 and 24 hrs of exposure, 25 µls of cell suspension were added to a reagent mix consisting of detergent to liberate nuclei, propidium iodide and RNase to serve as a pan-DNA dye, fluorescent antibodies against γH2AX, cleaved PARP, and phospho-H3, and counting beads for absolute cell (nuclei) counts. Data from the external labs were analyzed against a multinomial logistic regression-based model generated from the reference lab training set and the ability to correctly categorize the external test compounds as clastogen, aneugen or non-genotoxicant was determined. Values for concordance with a priori classification ranged from 75-100% across the six collaborating laboratories. Future work includes the examination of more compounds by the external labs as well as investigation of additional endpoints. These initial results are encouraging and suggest that a multiplexed and highly transferable technique may provide genotoxic mode of action information with remarkable efficiencies.

1061 Diet-Induced Obesity Increases the Frequency of Pig-a Mutations in Erythrocytes of Male Mice

M. Wilson[2], S. Dertinger[1], D. Torous[1], S. Avlasevich[1], B. Simon-Friedt[2] and J. Wickliffe[2]. *[1]Litron Laboratories, Rochester, NY and [2]Global Environmental Health Sciences, Tulane University, New Orleans, LA.*

Obesity increases the risk of a number of chronic diseases in humans including several cancers. Biological mechanisms responsible for such increased risks are not well understood at present. Increases in systemic inflammation and oxidative stress, endogenous production of mutagenic metabolites through microbiological processes or excess calorie and fat metabolism, altered signaling in proliferative pathways, and increased sensitivity to exogenous mutagens and carcinogens are likely at play. Therefore, we hypothesize that obesity alone creates an endogenously mutagenic environment in addition to increasing the sensitivity to environmental mutagens. To initially test part of our hypothesis, we examined somatic mutation frequencies in a mouse model of diet-induced obesity. Mutation frequencies in the Pig-a reporter gene were determined in blood cells (erythrocytes and reticulocytes) in 30-week old male mice reared on a high-fat diet (60% calories from fat) that exhibit an obese phenotype and matched mice reared on a normal-fat diet (10% calories from fat) that do not exhibit an obese phenotype. N-ethyl-N-nitrosourea (ENU) was used as a positive mutation control. ENU induced a robust mutation response with no apparent difference between phenotypes. However, untreated, obese mice had a significantly higher Pig-a gene mutation frequency (2.93-fold, $p<0.03$) in erythrocytes when compared to untreated non-obese mice. This suggests that obesity alone in the absence of an exposure to an exogenous mutagen is itself mutagenic.

1062 Dna Repair by MGMT and AAG in he Protection Against Colorectal Carcinogenesis

B. Kaina. *Department of Toxicology, University Medical Center, Mainz, Germany.* Sponsor: E. Fritsche.

Epidemiological studies indicate that N-nitroso compounds (NOC) are causally linked to colorectal cancer (CRC). NOC induce DNA alkylations, including O6-methylguanine (O6-MeG) and N-methylated purines, which are repaired by O6-methylguanine-DNA methyltransferase (MGMT) and N-alkyladenine-DNA glycosylase (AAG)-initiated base exci-

sion repair, respectively. In view of recent evidence of non-linear mutagenicity for NOC-like compounds on the one hand, and the importance of NOC for colon carcinogenesis on the other, the question arises as to the existence of thresholds in CRC formation. Here, we studied the impact of DNA repair on the dose-response of alkylation-induced CRC. DNA repair proficient (WT) and deficient (Mgmt-/-, Aag-/- and Mgmt-/-/Aag-/-) mice were treated with azoxymethane (AOM) and dextran sodium sulfate to trigger CRC. Tumors were quantified by non-invasive mini-endoscopy. A non-linear increase in CRC formation was observed in WT and Aag-/- mice. In contrast, a linear dose-dependent increase in tumor frequency was found in Mgmt-/- and Mgmt-/-/Aag-/- mice. The data was corroborated by hockey stick modeling, yielding similar carcinogenic thresholds for WT and Aag-/- and no threshold for MGMT lacking mice. O6-MeG levels and depletion of MGMT correlated well with the observed dose-response in CRC formation. AOM induced DNA double-strand breaks in colon crypts, including Lgr5-positive colon stem cells, and triggered ATR-Chk1-p53 signaling. Intriguingly, Mgmt-/- mice displayed significantly enhanced levels of γ-H2AX, suggesting the usefulness of γ-H2AX as an early genotoxicity marker in the colorectum. The study revealed that DNA repair by MGMT, but not AAG, protects at low AOM dose levels against colorectal carcinogenesis and, therefore, is a key node in determining a carcinogenic threshold.

PL 1063 Innate Lymphoid Cells Mediate Type 2 Immunity in the Lungs of Mice Repeatedly Exposed to Ozone

K. M. White[1], J. Harkema[2] and K. Kumagai[2]. *[1]Biology, Spelman College, Atlanta, GA and [2]Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI.*

High levels of ambient ozone, a common air pollutant, are associated with increased incidences of eosinophilic rhinitis and asthma in children. Repeated ozone exposures also cause eosinophilic rhinitis and nasal epithelial remodeling in mice. We have found that these nasal lesions are dependent on innate lymphoid cells (ILCs). The role of ILCs in ozone-induced pulmonary toxicity has not been previously investigated. In the present study, we determined the ILC-dependency of lung pathology caused by acute (1-day) and subacute (9-day) ozone exposure. Lymphoid cell-sufficient C57BL/6 mice, Rag2-/- mice devoid of T and B lymphoid cells (but ILC sufficient), and Rag2-/-Il2rg-/- mice, devoid of all lymphoid cells (including ILCs) were exposed to 0 or 0.8 ppm ozone for 1 or 9 day(s) (4 h/day). Bronchoalveolar lavage fluid (BALF) was collected for inflammatory cell content and lung tissues were processed for histopathology. After acute ozone exposure, all mouse strains (ILC-sufficient and -deficient) had similar acute airway injury characterized by increased BALF neutrophils and bronchiolar epithelial cell proliferation in response to toxicant-induced cell death. After subacute exposure only ILC-sufficient mice had lung pathology that included increases of BALF eosinophils and lymphocytes, and mucous cell metaplasia of bronchiolar epithelium (type 2 immune responses). These results indicate that lung lesions in mice caused by acute ozone exposure are ILC independent, but the type 2 immune lung lesions induced by subacute exposure are ILC dependent. This study provides a plausible biological mechanism for underlying epidemiological associations of high ambient ozone and childhood asthma.

PL 1064 Scavenger Receptor BI Regulates Pulmonary Inflammation after Ozone Exposure

R. M. Tighe[1], A. Birukova[1], M. J. Yaeger[4], B. Luo[4], M. B. Fessler[2], C. J. Wingard[4] and K. M. Gowdy[4]. *[1]Duke University, Durham, NC; [2]Nathional Institutes of Environmental Health Sciences, Research Triangle Park, NC; [3]Pharmacology and Toxicology, East Carolina University, Greenville, NC and [4]Physiology, East Carolina University, Greenville, NC.*

Rationale: Ozone (O3) exposure is associated with increased cardiopulmonary-induced morbidity and mortality. O3 does not have direct effects on the pulmonary and cardiovascular system; rather it generates modified lipids and proteins in the lung. These modified products bind and stimulate pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and scavenger receptors (SRs). Scavenger Receptor B-I (SR-BI) is a multi-recognition receptor critical in cellular uptake of cholesterol ester from high density lipoprotein. Recently, we reported that SR-BI plays a role in the pulmonary immune response to lipopolysaccharide (LPS) but the contribution of SR-BI to O3 induced lung injury is unknown. Methods: C57Bl/6J (WT) and SR-BI-/- mice were exposed to 2ppm of O3 or filtered air for 3 hours to determine pulmonary injury and immune responses. Neutral lipid loading of alveolar macrophages (Oil Red O staining), bronchoalveolar lavage (BAL) cytokines, and differentials were quantified 12hrs after exposure. In WT mice, SR-BI gene expression was also assessed in whole lung and pulmonary macrophages isolated from the airspace at 0, 3, 6, 12, and 24hrs post exposure. Results: Real time PCR analysis of SR-BI expression in WT lung tissue revealed that SR-BI is significantly induced about 3-6 hours post O3 exposure but returns to baseline levels by 24 hrs post O3 exposure. A similar trend for SR-BI expression was seen in pulmonary macrophages. Compared to WT counterparts, SR-BI-/- mice developed higher levels of neutrophils, IL-17, TNF-α, IL-6, and G-CSF in the airspace after O3 exposure, but had equivalent amounts of MIP-2, KC, and macrophages. Protein was also increased in the BAL of SR-BI-/- mice 12hrs after O3 exposure indicating increased microvascular injury. Additionally, SR-BI-/- alveolar macrophages had increased lipid loading after O3 when compared to WT mice. Conclusions: SR-BI expression is induced in the lung and macrophages after O3 exposure. This receptor is critical in modulating the pulmonary response to O3 exposure by regulating pulmonary injury, macrophage lipid uptake, and neutrophil influx. Together, these data suggest a novel protective role for SR-BI during adverse health effects of ozone exposure.

PL 1065 Comparative Toxicity of Simulated Smog Atmospheres in Healthy and Allergic Mice

M. McGee[1], L. Copeland[2], Q. Krantz[2], C. King[2], J. Krug[2], C. Wood[2], M. Gilmour[2] and S. Gavett[2]. *[1]Oak Ridge Institute for Science and Education, Oak Ridge, TN and [2]Office of Research and Development, US EPA, Research Triangle Park, NC.*

Effects of complex regional multipollutant mixtures on disease expression in susceptible populations are dependent on multiple exposure and susceptibility factors. Differing profiles of ozone (O3), nitrogen dioxide (NO2), and particulate matter (PM), which are key components of smog, and other hazardous pollutants may develop as a result of regional-specific geographic and urban environment characteristics. We investigated the pulmonary effects of two smog mixtures with different compositions in a mouse model of allergic airway disease to determine which source profile had the greatest impact on pulmonary endpoints. A hydrocarbon mixture was combined with NO gas in the presence of UV light in a controlled setting. Simulated smog atmosphere 1 (SSA-1) consisted of concentrations of 1070 µg/m3 secondary organic aerosol (SOA), 0.104 ppm O3, and 0.252 ppm NO2. SSA-2 consisted of a starting concentration of 53 µg/m3 SOA, 0.376 ppm O3, and 0.617 ppm NO2. SOA concentration of SSA-2 increased upon delivery to the exposure chamber. Healthy and house dust mite (HDM)-sensitized (allergic) female BALB/cJ mice were exposed 4 hr/day for 1 or 5 days to either smog mixture or clean air. Two days after HDM challenge, airway mechanics were tested in anesthetized ventilated mice. Following methacholine aerosol challenge, increased airway resistance and elastance and a decrease in lung compliance were consistently observed in air- and smog-exposed HDM-allergic groups compared with non-allergic groups. Neither SSA exposure produced significant changes in lung injury markers (bronchoalveolar lavage protein, lactate dehydrogenase). Further analyses will be conducted to investigate effects of allergic challenge or smog exposure on nasal and lung histopathology. Generation of simulated smog atmospheres in a photochemical reaction chamber allows precise control of atmospheric chemistry for real-time exposures, but to date few significant changes in health endpoints have been observed in the mouse allergy model. (This abstract does not represent US EPA policy.)

PL 1066 Coconut, Fish, and Olive Oil-Rich Diets Modify Ozone-Induced Metabolic Effects

S. J. Snow[1,3], H. Tong[3], D. Miller[2], M. Schladweiler[3], J. Richards[3], D. Andrews[3] and U. Kodavanti[3]. *[1]ORISE, Durham, NC; [2]University of North Carolina Chapel Hill, Chapel Hill, NC and [3]US EPA, Durham, NC.*

Pulmonary health effects of ozone (O3) exposure are well known; however, the cardiovascular and metabolic consequences are still under investigation. Fish oil (FO) and olive oil (OO) dietary supplementation have several cardioprotective benefits, but it is not established if these supplements can protect against the adverse pulmonary and metabolic effects induced by exposure to air pollution. We hypothesized that dietary supplementation with FO and OO would attenuate O3-induced metabolic impairments. Male Wistar Kyoto rats were fed either a normal diet, or a diet enriched with FO, OO, or coconut oil (CO; a control oil diet) starting at 4 weeks of age. The CO and OO diets increased body fat % as well as serum triglyceride and total cholesterol levels as compared to the animals fed the normal and FO diets. Serum leptin, a satiety hormone, was elevated with all dietary supplementation; however, glucose tolerance testing (GTT) indicated that blood glucose levels and glucose tolerance were unaffected by the oil-based diets. Eight weeks following the initial start of the diet, animals were exposed to air or 0.8 ppm O3, 4h/day for 2 days. GTT illustrated O3-induced hyperglycemia and glucose intolerance regardless of diet, with further exacerbation of glucose

intolerance occurring when animals were fed the OO diet. O3 exposure significantly increased total cholesterol, LDL cholesterol, leptin, and branched chain amino acids in animals fed the normal diet, indicating metabolic impairment. The various oil-enriched diets selectively attenuated these responses with FO being the only supplement able to abolish all O3-induced increases in these metabolic biomarkers. Collectively, these data indicate that FO offers protection against metabolic impairment induced by an acute O3 exposure, and suggest that this simple dietary supplementation will be beneficial for individuals that reside in highly polluted regions. (This abstract does not reflect US EPA Policy).

PL 1067 Ozone-induced Pulmonary Injury and Vascular Contractility Are Differentially Impacted by Coconut, Fish, and Olive Oil-Rich Diets

M. C. Schladweiler[3], S. Snow[1,3], H. Tong[3], V. Bass[1,2], J. Richards[3], R. Jaskot[3] and U. Kodavanti[3]. *[1]ORISE, Research Triangle Park, NC; [2]UNC, Chapel Hill, NC and [3]US EPA, Durham, NC.*

Pulmonary and systemic effects of ozone (O3) are mediated by hypothalamic-pituitary-adrenal axis activation. Fish oil (FO) and olive oil (OO) dietary supplementation have cardioprotective benefits, but it is not established if these supplements protect against the adverse pulmonary and vascular effects induced by exposure to air pollution. We hypothesized that FO and OO diets will decrease O3-mediated pulmonary injury/inflammation and vascular contractility. Male Wistar Kyoto rats were fed a normal diet or a diet enriched with FO, OO, or coconut oil (CO - a control oil diet) starting at 4 weeks of age. Eight weeks following the start of diet, animals were exposed to air or 0.8 ppm O3, 4h/day for 2 days. The CO and OO diets increased serum triglyceride and total cholesterol levels as compared to the animals fed the normal and FO diets. FO diet increased baseline levels of bronchoalveolar lavage fluid (BALF) protein, lactate dehydrogenase and n-acetyl glucosaminidase activity (NAG), as well as inflammatory cells (macrophages, neutrophils and eosinophils) but did not increase albumin leakage. O3-induced increase in BALF protein was exacerbated in rats fed OO relative to FO, however albumin leakage was least affected by FO diet. O3 increased NAG regardless of diet; this increase was further exacerbated in rats fed all lipid-supplemented diets relative to normal chow. O3 increased neutrophilic inflammation in all dietary groups except for FO, whereas eosinophilic inflammation was evident in only the CO and OO dietary groups. O3-induced drop in circulating white blood cells was least affected in rats fed FO diet. FO supplementation protected against phenylephrine-induced vasoconstriction in thoracic aortic ring segments that was present in all other O3 exposed rats. Collectively, these data indicate that FO, while increasing BALF protein content and inflammation at baseline, offers protection from O3-induced vascular albumin leakage in the lung and aortic vasoconstriction. (Does not reflect the US EPA policy).

PL 1068 Active versus Sedentary Lifestyle from Childhood to Adult and Susceptibility to Ozone: An Animal Model

C. J. Gordon, P. M. Phillips, A. F. Johnstone, A. Ledbetter, M. C. Schladweiler, S. J. Snow and U. P. Kodavanti. *US EPA, Research Triangle Park, NC.*

A pattern of sedentary lifestyle beginning in childhood is associated with obesity and related disorders such as type 2 diabetes. Obesity is associated with increased susceptibility to air pollutants and initiating regular exercise early in life should impact positively on respiratory symptoms of air pollutant exposure. An animal model of childhood-to-adult sedentary (SED) vs. active (ACT) lifestyle was achieved by providing female Long-Evans rats with running wheels beginning at 22 d of age and then exposing to ozone (O3) as adults. ACT rats ran 7.2 km/d over 74 days, had lower body fat, and improved glucose tolerance (GT) compared to SED rats. Adult rats were exposed to 0, 0.25, 0.5, or 1.0 ppm O3 for 5 hr/d for 2 d. O3-induced impairment in GT was significantly improved in ACT animals. Bronchoalveolar lavage (BALF) protein markers of lung damage and neutrophilic inflammation were similarly affected in SED and ACT animals. BALF eosinophils of SED rats were markedly higher after exposure to 0.5 and 1.0 ppm O3 compared to ACT rats. Overall, this animal model suggests that regular exercise initiated early in life may afford protection in adulthood to the metabolic and pulmonary effects of O3. The attenuated O3-induced elevation in BALF eosinophils of ACT rats may suggest a protective mechanism of childhood exercise on asthma-related symptoms of air pollution. This is an abstract of a proposed presentation and does not reflect US EPA policy.

PL 1069 Acute Ozone-Induced Pulmonary and Systemic Metabolic Effects Are Diminished in Adrenalectomized Rats

D. B. Miller[1], M. C. Schladweiler[3], S. J. Snow[3], J. E. Richards[3], D. L. Andrews[3], A. J. Ghio[3], A. D. Ledbetter[3] and U. P. Kodavanti[3]. *[1]Curriculum in Toxicology, University of North Carolina Chapel Hill, Durham, NC; [2]Environmental Public Health Division, US EPA, Research Triangle Park, NC and [3]Proteomics Core, National Health and Environmental Effects Research Laboratory, US EPA, Research Triangle Park, NC.*

Acute ozone exposure increases circulating stress hormones and induces peripheral metabolic alterations in animals and humans. We hypothesized that the increase of adrenal-derived stress hormones is necessary for ozone-induced systemic metabolic effects and lung injury. Male Wistar-Kyoto rats (12 week-old) underwent total bilateral adrenalectomy (ADREX), adrenal demedullation (DEMED) or sham surgery (SHEM). After a 4 day recovery, rats were exposed to air or ozone (1ppm), 4h/day for 1 or 2 days. Circulating adrenaline levels dropped to nearly zero in DEMED and ADREX rats relative to SHAM. Corticosterone levels tended to be low in DEMED rats and dropped to nearly zero in ADREX rats. Adrenalectomy in air-exposed rats caused modest changes in metabolites and lung toxicity parameters. Ozone-induced hyperglycemia and glucose intolerance were markedly attenuated in DEMED with nearly complete reversal in ADREX rats. Ozone increased circulating epinephrine and corticosterone in SHAM but not in DEMED or ADREX rats. Free fatty acids and branched-chain amino acids tended to increase after ozone exposure in SHAM but not in DEMED or ADREX rats. Lung minute volume was not affected by surgery or ozone; however, ozone-induced labored breathing was less pronounced in ADREX rats. Ozone-induced increases in lung protein leakage and neutrophilic inflammation were markedly reduced in DEMED and ADREX rats (ADREX>DEMED). Ozone-mediated decreases in circulating WBC in SHAM was not observed in DEMED and ADREX rats. We show that ozone-induced peripheral metabolic effects and lung injury/inflammation are mediated through adrenal-derived stress hormones as a result of activation of the stress response pathway. (Does not reflect US EPA Policy).

PL 1070 Inhalation of Simulated Smog Affects Cardiac Function in Mice

H. Tong[1], Q. Krantz[2], M. McGee[2], J. Krug[2], C. King[2] and S. Gavett[2]. *[1]US EPA, Chapel Hill, NC and [2]US EPA, Research Triangle Park, NC.*

Rationale: The health effects of individual criteria air pollutants have been well investigated. Little is known about health effects of inhaled multi-pollutant mixtures that more realistically represent environmental exposures. The present study was designed to evaluate the cardiac effects of inhaled simulated smog mixture in a murine model. Methods: Four-month old female C57/Bl6J mice were exposed for 4 hours to filtered air (FA) or simulated smog generated by mixture of 15 ppm hydrocarbon and 900 ppb NO gas in the presence of UV light. Final exposure conditions consisted of 53 µg/m3 secondary organic aerosol (SOA), 0.376 ppm O3, and 0.617 ppm NO2. Immediately and 20 hr after exposure, cardiac responses were assessed with a Langendorff preparation using a protocol consisting of 20 min of global ischemia followed by 2 hr reperfusion. Cardiac function was measured by index of left-ventricular developed pressure (LVDP) and contractility (dP/dtmax and dP/dtmin) before ischemia. On reperfusion after ischemia, recovery of post-ischemic LVDP and infarct size were examined. Results: Baseline LVDP and dP/dtmax were lower in the mice 20 hr after the simulated smog exposure compared to the FA control. Baseline LVDP was also decreased immediately after smog exposure. However, there was no statistically significant difference of post-ischemic recovery of cardiac function and infarct size between the smog and FA groups at either time points. In addition, smog exposure reduced the coronary artery flow rate compared to the FA control. Heart rate was not affected by the exposure. Conclusions: This study demonstrated that acute smog exposure decreases baseline LVDP, cardiac contractility, and coronary flow rate in mice, indicating that photochemically-altered atmosphere affects the cardiovascular system. (This abstract does not necessarily reflect US EPA policy.)

1071 Changes in DNA Methylation Associated with Acute Ozone Exposures in Healthy Human Subjects

J. Mirowsky[2], M.-A. Bind[5], B. Coull[4], Z. Liu[4], J. Schwartz[4], D. Diaz-Sanchez[6], K. Duncan[2] and R. Devlin[6]. *[1]Center for Environmental Medicine, Asthma, and Lung Biology, University of North Carolina, Chapel Hill, NC; [2]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC; [3]Department of Biostatistics, Harvard University, Boston, MA; [4]Department of Environmental Health, Harvard University, Boston, MA; [5]Department of Statistics, Harvard University, Cambridge, MA and [6]Environmental Public Health Division, US EPA, Chapel Hill, NC.*

Ozone, an important component to air pollution, has been associated with airway inflammation, respiratory diseases, and mortality. However, the mechanisms responsible for these adverse outcomes has not yet been fully elucidated. Previous studies have shown changes in DNA methylation associated with ambient pollution exposures, suggesting DNA methylation may serve as mechanistic support for adverse responses; however, no work has been conducted under controlled conditions. In this study, we assessed whether changes in epigenome-wide DNA methylation are observed in healthy human subjects after acute exposures to 300 ppb ozone. Using a randomized crossover experiment of 17 volunteers, subjects were exposed on two occasions (i.e., once to clean air and once to ozone) for two hours while exercising intermittently. Twenty hours after exposure, participants underwent a bronchoscopy, during which bronchial epithelial cells were removed via brush biopsy. Using these cells, which are among the first targets of inhaled pollutants, epigenome-wide DNA methylation was measured with Illumina HumanMethylation 450K BeadChips. The methylation data was pre-processed and corrected for background, dye bias, and probe design bias. Significant changes in percent methylation were found in several genes when comparing clean air exposures to ozone. Specifically, when participants were exposed to clean air and ozone, mean methylation in FAS, a gene related to programmed cell death, was equal to 0.07%mC and 0.10%mC, respectively. Additionally, IL-1F8, which is related to inflammation, had mean methylation values of 0.10%mC and 0.14%mC for clean air and ozone. The results from this study suggest causal effects of ozone on the human epigenome can be measured in this healthy population.

1072 Bioinformatics Choices Substantially Impact Isoform Analysis of RNA-Aeq Data from a Toxicogenomics Study

J. Z. Xu[2], X. Chen[1], S. Subramani[2], B. Gong[2] and W. Tong[2]. *[1]Department of Histology and Embryology, Harbin Medical University, Harbin, Heihongjiang, China and [2]Division of Bioinformatics and Biostatistics, National Center for Toxicological Research, Jefferson, AR.*

Alternative splicing events greatly increase the diversity of proteins which might response differently to toxic insults. Identification and quantification of isoforms are important for toxicological research for an improved understanding of the underlying mechanisms of toxicity. The advent of RNA-seq technology and related bioinformatics tools enable detection of novel isoforms and quantification of relative transcript abundances and thus enhance the research of alternative splicing. However, recent works have shown highly inconsistent results of quantitative analysis and there is no clear understanding of the source of variation. In this study, we investigated the impact of five potential factors on isoform detection and quantification, including the choice of bioinformatics methods, sequencing depth, library preparation, transcript abundance and treatment effect. Liver RNA samples from six rats (three treated by aflatoxin B1 for 5 days and three matched controls) were profiled with two libraries prepared for each sample. The first batch of libraries was sequenced twice while the second batch sequenced only once. Five bioinformatics pipelines were used, with two mapping tools (TopHat2 and STAR) followed by three isoform analysis approaches (Cufflinks, IsoLasso and FlipFlop). We evaluated the consistency of isoform detection for each potential factor and compared the differential analysis results of isoform expression and splicing for each factor. In summary, the choice of bioinformatics pipelines has a substantial impact on all aspects of quantitative isoform analysis of RNA-seq data, including the number of isoforms detected, differentially expressed isoforms, and their low concordance (about 41% on average) between pipelines. Differentially used transcription starting sites or alternative splicing events for the short-term aflatoxin B1 treatment cannot be reliably detected.

1073 Utilizing RNA-Seq to Increase the Sensitivity of a Micropatterned Human Hepatocyte Co-culture Platform (HepatoPac®) in Identifying Human Drug Induced Liver Injury

P. Koza-Taylor[2], S. Mathur[2], O. Ukairo[1], O. Irrechukwu[1], M. Lawton[2] and M. Aleo[2]. *[1]Hepregen, Medford, MA and [2]Pfizer, Groton, CT.*

Drug-induced liver injury (DILI) in humans can result in termination of drug development or withdrawal from the market post approval. Current platforms utilized for compound screening early in drug development do not accurately predict whether a compound will cause DILI in the clinic. These platforms typically rely on classic biomarkers secreted into the media or released in the serum that lack specificity/sensitivity. This study was performed in order to determine if molecular processes can be identified *in vitro* that would be able to identify compounds with DILI risk in humans. The HepatoPac platform, comprised of a co-culture of mouse 3T3 J2 fibroblasts and human primary hepatocytes, was dosed with two peroxisome proliferator-activated receptor (PPAR)γ/α activators, troglitazone (TGZ) and pioglitazone (PGZ). RNA was isolated from the mixed-species cell population and RNA-Seq data were generated. The combination of human and mouse sequences were separated using Xenome and resulted in ~55% human and ~42% mouse sequences. Differential expression analysis of the human sequences yielded ~ 900 differentially expressed genes (DEGs) for TGZ and PGZ at 10% FDR with fold change > |1.5|. The DEGs from TGZ treatment mapped to significantly more pathways and processes than the similar number of DEGs from PGZ treatment. Pathways indicative of pharmacology (i.e., an upregulation of PPARα signaling) were seen with both compounds. In addition TGZ treatment resulted in the identification of processes related to endoplasmic reticulum/oxidative stress and inflammation, processes that have previously been associated with DILI risk (Laifenfeld et al., 2014). Only a weak inflammatory signal was identified for PGZ treatment. The transcriptional profiles were able to differentiate DILI-causing compound TGZ from the DILI negative compound, PGZ. These results support the application of RNA-Seq/pathway analysis in the HepatoPac model to identify compounds with clinical DILI risk.

1074 Evaluation of the Literature Prioritization Capabilities of SWIFT-Review, a Tool for Conducting Systematic Reviews of Environmental Health Questions

B. E. Howard[2], K. Miller[2], J. Phillips[2], K. Thayer[1] and R. Shah[2]. *[1]National Institute of Environmental Health Sciences, Research Triangle Park, NC and [2]Sciome, LLC, Research Triangle Park, NC.*

During the problem formulation stage of a chemical-centric systematic review, it is typical for environmental toxicologists to conduct complex searches of the scientific literature which may yield thousands or tens of thousands of potentially relevant documents. In collaboration with NIEHS, we have developed the SWIFT-Review software, a freely available application that can assist researchers in prioritizing, filtering and organizing these large literature search results. Here we report the results of an evaluation of the literature prioritization module of our software. Given the annotated results of 20 different manually curated literature searches, we used SWIFT-Review to train and evaluate statistical models of the relevant literature. At the 95% recall level, the work saved over random sampling (WSS@95) exceeded 70% for 7 out of 20 of these datasets, and was more than 50% on average for all 20 datasets. These results indicate that the use of this method has the potential to reduce by more than half the screening burden required to achieve 95% recall of the relevant literature. By integrating this document triage capability together with topic modeling, ad-hoc search functionality, and automatic tagging of chemical names, environmental exposures, evidence stream and health effects, SWIFT-Review provides a valuable set of tools useful for anyone interested in efficiently navigating large collections of toxicological literature.

1075 Using Literature Network Analysis to Evaluate Information Gained from ToxCast Chemical Screening

S. Watford[2] and M. Martin[2]. *[1]US EPA, Research Triangle Park, NC and [2]NCCT, US EPA, Research Triangle Park, NC.* Sponsor: M. Martin.

Characterizing the effects of chemicals in biological systems is often summarized by chemical-gene interactions, which have sparse coverage in the literature. The ToxCast chemical screening program has produced bioactivity data for nearly 2000 chemicals and over 450 gene targets. To evaluate the information gained from the ToxCast project, a ToxCast

bioactivity network was created comprising ToxCast chemical-gene interactions based on assay data and compared to a chemical-gene association network from literature. The literature network was compiled from PubMed articles, excluding ToxCast publications, mapped to genes and chemicals. Genes were identified by curated associations available from NCBI while chemicals were identified by PubChem submissions. The frequencies of chemical-gene associations from the literature network were log-scaled and then compared to the ToxCast bioactivity network. In total, 140 times more chemical-gene associations were present in the ToxCast network in comparison to the literature-derived network highlighting the vast increase in chemical-gene interactions putatively elucidated by the ToxCast research program. There were 165 associations found in the literature network that were reproduced by ToxCast bioactivity data, and 336 associations in the literature network were not reproduced by the ToxCast bioactivity network. The literature network relies on the assumption that chemical-gene associations represent a true chemical-gene interaction. This bias along with other confounders and known issues (e.g., term mapping) that will be better accounted for in future versions of the literature network with additional manual curation or natural language processing methods applied. Meanwhile, the comparison of the ToxCast and literature networks demonstrated the increase in chemical-gene associations being discovered through screening programs and the need to improve chemical and gene annotations in the literature. This approach provides a framework for estimating information gain from ToxCast and other chemical screening programs as well as highlighting areas of follow-up to confirm chemical-gene interactions previously unreported in the literature. This abstract does not necessarily reflect US EPA policy.

1076 Elucidating the Molecular Interactions of PTEN Tumor Supressor

S. Yeguvapalli and K. N. Chitrala. *Zoology, Sri Venkateswara University, Tirupati, India.*

Phosphatase and tensin homolog (PTEN) is a highly studied tumour suppressor gene. PTEN mutations are identified in many sporadic tumours and it is known to be commonly mutated in triple-negative breast cancers. Bisperoxovanadium (bpV) compounds are known to be irreversible protein tyrosine phosphatase (PTP) inhibitors with a spectrum of activity distinct from the vanadium salts. These bpV compounds are known to interact pharmacologically with PTEN by binding to the CX5R motif, shared by PTPs. In the present study, we aim to analyze the interactions of bpV with PTEN tumor suppressor. Crystal structure of PTEN tumor suppressor was downloaded from the pdb database. Molecular docking was performed using Autodock followed by the molecular dynamic simulations. Results showed that a large deviation in the total solvent accessible surface area, structure solvent energy and average gain in the bpV bound PTEN tumor suppressor compared to its unbound form. Based on these results, we conclude that the interaction of bpV induces conformational changes in the PTEN tumor suppressor (Supported by UGC Research award 2014-16).

1077 Abstract Withdrawn by Author.

1078 An Adaptive Method for Normalization of MicroRNA Array Data

Y. Zhu[3], Q. Zhao[1], K. Staflin[3], D. Sheinson[3], M. Sweeney[3], J. Tarrant[3], W. Proctor[3], J. Vogt[3] and M. Tseng[3]. [1]*Department of Biostatistics, Yale University, New Haven, CT;* [2]*Nonclinical Biostatistics, Genentech, Inc., South San Francisco, CA and* [3]*Safety Assessment, Genentech, Inc., South San Francisco, CA.*

MicroRNAs (miRNAs) are known to play important regulatory roles in many cellular processes. Currently, there is no consensus on an optimal normalization strategy for qRT-PCR miRNA quantification analysis, and yet rigorous normalization of miRNA data can be critical since relatively small changes in miRNA expression may be biologically and clinically significant. We propose MiRA-norm (MiR-Adaptive normalization method) to construct an adaptive set of housekeeping miRNAs that are stable in expression level and capture the systematic patterns induced by sample preparation and other technical artifacts. We illustrate the advantages and practical issues involved in implementing the proposed method across a variety of scenarios through the following case studies: 1) rat and human serum samples obtained for the detection of miR-122, a tissue-specific miRNA that is transcriptionally regulated in the liver for which circulating levels have been associated with drug-induced liver injury, viral hepatitis, and autoimmune hepatitis, 2) a neuroblastoma tumor sample set designed to detect differentially expressed miRNAs between MYCN single copy and MYCN amplified tumor samples with known positive miRNAs confirmed by ChIP-chip experiment, and 3) a biomarker assessment of tissue specific toxicity in a preclinical setting with molecule A. Our results demonstrate that adaptive normalization is promising as a robust normalization strategy.

1079 Network Analysis on ToxCast Data

H. Ye, H. Luo, H. Ng, S. D. Sakkiah, W. Tong and H. Hong. *US FDA, National Center for Toxicological Research, Jefferson, AR.* Sponsor: W. Tong.

Most so called "big datasets" are also incomplete, with varying degrees of scarcity. Denoting such datasets as sparse datasets for convenience, they pose challenges for traditional machine learning and classification algorithms. Thus, there is a need to develop novel approaches to deal with sparse and big datasets in toxicological research. We developed the Neighbor-Edges Based and Unbiased Leverage Algorithm (NEBULA) which is specifically to address this challenge. The US Environmental Protection Agency's (EPA) ToxCast project evaluated a diverse set of chemicals, including both environmental chemicals and drugs, using a broad panel of high-throughput *in vitro* assays. ToxCast data have been studied to characterize the toxicological profiles of environmental chemicals. However, the dataset has a high degree of missing elements and thus is sparse. In this investigation, we applied NEBULA and modularity analysis for ToxCast data. We constructed the chemical-assay network that consisted of 32,763 data points with 1,799 chemicals and 544 assays and decomposed the network into seven densely connected modules based on its topological properties. We found that each of the seven modules was associated with different set of adverse outcome pathways (AOPs) as well as chemical structural descriptors. Leave-one-out cross validations showed a high consistency between experimental AC50 values from ToxCast and predicted AC50 values from NEBULA with a Q2 of 0.542. Our study demonstrated NEBULA to be an efficient algorithm for analyzing sparsely populated big data which has become common in the toxicological research. Our results also indicated ToxCast data could be used to obtain toxicological profile of chemicals that have not been assayed in ToxCast for risk assessment.

1080 Inferring Transcriptional Regulatory Networks for Alzheimer's Insights: Brain *In Situ* Hybridization Maps as an Alternative to Microarrays

G. K. Acquaah-Mensah[2] and R. C. Taylor[1]. [1]*Computational Biology & Bioinformatics Group, Pacific Northwest National Laboratory, Richland, WA and* [2]*Pharmaceutical Sciences, Massachusetts College of Pharmacy and Health Sciences, Worcester, MA.*

Microarray data have been a valuable resource for identifying transcriptional regulatory relationships (TRRs) among genes. TRRs most relevant to diseases such as Alzheimer's Disease (AD) are brain region-specific, limiting the utility of TRRs identified on the basis of whole brain microarrays. Often, there is a paucity of suitable brain region-specific microarrays for the purpose of network inference. Here, we explore the Allen Brain Atlas mouse *in situ* hybridization (ISH) data as a plausible alternative source of high throughput brain region-specific gene expression data. Further, we explore the contributions of glucose and dietary fats to the AD etiology using Transcriptional Regulatory Networks (TRNs). The hippocampus in AD is particularly important because of its association with learning and memory. In this study, ISH data in the hippocampus regions (per the Allen Bran Atlas) were extracted, focusing on genes relevant to neurodegeneration. TRNs were learned using high-performing network inference algorithms: Context Likelihood of Relatedness (CLR), GEne Network Inference with Ensemble of trees (GENIE3), and Inferelator. A human post-mortem hippocampal microarray AD data set was analyzed, and the results superposed on the networks inferred (orthologs of differentially expressed genes were used). Ncor2, Nfe2l1, Gcm2, Sp3, Egr1 and Usf2 emerge among notable regulators of several genes known to be involved in AD (such as Dhcr24, Aplp2, Tia1, Pdrx1, Vdac1, and Syn2), further elucidating their functions. Ncor2, Sp3 and Usf2 form a unique three-party regulatory motif, affecting memory formation pathways. Further, Nfe2l1, Sp1 (which is highly homologous to Sp3), Egr1 and Usf2 are all sensitive to dietary factors and could be among links between dietary influences and genes in the AD etiology. Thus, this approach of harnessing brain region-specific ISH data represents a unique opportunity for gleaning unique etiological insights for diseases such as AD.

1081 Metabolomics Investigation of Xenobiotics and Emtabolic Pathway Networks in Tumors, Adjacent Mucosa, and Stool from Colorectal Cancer Patients

D. G. Brown and E. P. Ryan. *ERHS, CSU, Fort Collins, CO.*

Colorectal cancer (CRC) is the third leading cause of cancer-related death in the United States. Little is known regarding the profile of xenobiotics that may influence CRC progression across colonic tumor locations in people. We hypothesized that xenobiotic metabolites and pathways differ in both their presence and relative abundance in tumor tissue and adjacent mucosa when compared to detection in stool and across tumor locations. 33 tissue (16 tumor tissue and 17 patient-matched mucosa) and 13 stool samples were collected from CRC patients undergoing colonic resection and analyzed by Liquid Chromatrography-Mass Spectrometry and Gas Chromatography-Mass Spectrometry to determine global metabolite profiles (Metabolon, Inc). Normalized relative intensity values for xenobiotics detected were uploaded into MetaboAnalyst and Metabolon's Metabolync pathway analysis software to determine metabolic pathways affected. Matched-pairs and student's t-tests were used to evaluate differences between metabolite expression in tumors and mucosa and differences between tumor locations in stool and tumor tissue matrices. Of the 703 metabolites in this dataset, 86 were xenobiotics that included 6 benzoate, 14 chemical, 19 drug, 36 food/plant components, and 11 xanthine metabolites across all human tissue and stool samples analyzed. There were 15 xenobiotics specific to tissue, 50 unique to stool, and 21 detected in both. Of the shared metabolites, quinate had the greatest fold difference (2.86) between tumor tissue and mucosa. The following drugs (ofloxacin and probenecid), plant/food components (enterolactone and caffeate) and chemicals (tetraethylene glycol and brilliant blue FCF) were identified in stool with substantial variation in their relative abundance. Metabolic pathway analysis of xenobiotics further revealed modulation of caffeine metabolism with 5 shared metabolites across matrices affected, including paraxanthine, 1,7-dimethyluric acid, caffeine, 1,3,7-trimethyluric acid, and theobromine. The xenobiotic profiles identified using metabolomics showed novel relationships between sub-metabolic pathways shared between tumor tissue and mucosa as well as with distinctions in stool. This finding increased our knowledge of xenobiotic profiles and networks in CRC patients and provides information about the tumor microenvironment with regards to compounds absorbed by colonic tumors and mucosa when compared to excretion in stool.

1082 Novel Computational Approaches for High Content Image Analyses (HCA) of Organoid Neurosphere Cultures *In Vitro*

M. R. Schmuck[2], T. Temme[1], M. Barenys[2], A. Mosig[1] and E. Fritsche[2]. *[1]Bioinformatics Group, Ruhr University Bochum, Bochum, Germany and [2]Modern Risk Assessment & Sphere Biology, IUF-Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany.*

Neurospheres are three dimensional (3D) cell clusters consisting of neural progenitor cells (NPCs). NPCs provide the cellular basis for the developing brain and persist in the hippocampus up to old age. There, they are responsible for adult learning and memory. Safety and efficacy testing with NPCs growing as 3D neurospheres by employing high content image analyses (HCA) requires advanced scanning and evaluation processes which is due to characteristic features of the culture: 1) A 3D sphere core, which leads to unfocused images, 2) a variable cell density within the migration area, 3) a heterogonous cell population of neurons and glia cells and 4) sphere-specific endpoints. Therefore, for this mixed neuron/glia culture we developed algorithms for the quantification of neuronal differentiation, neurite outgrowth, radial migration and distance-dependent density distributions of neurons. For quantification of neuronal differentiation the new algorithm reaches an average detection power (DP) of 80–85% (versus manual evaluation) and a false positive rate (FP) of 10–15% improving results of the commercially available 'Neuronal Profiling' bio-application (Thermo Scientific; DP: 50%, FP: 40%), which was initially designed for pure neuronal cultures. For evaluation of the neurosphere-specific endpoint 'radial cell migration' our self-developed program recognizes the sphere core as a single object and determines migration distance as an average of the distances between the rim of the sphere core and all identified nuclei coordinates with the furthest migration distances in all directions. Another self-written algorithm quantifies positioning of individual cell types within the migration area leading to a distance-dependent density distribution. In conclusion, HCA of neurospheres is a promising technique for medium throughput screening to be used in safety and efficacy testing in the future.

1083 Predicting Chemical-Induced Human Organ Injuries Based on Gene Expression Changes Caused by Chemical Treatment of Cultured Human Cells

R. Liu, X. Yu and A. Wallqvist. *DoD Biotechnology High Performance Computing Software Applications Institute, Frederick, MD.*

It is well known that animals and humans may have different responses to chemical exposures, but dosing animals with chemicals remains a common practice to predict toxicity to humans. In this study, we investigated the feasibility of predicting chemical toxicity to humans based on chemically induced gene expression changes in cultured human cells. Our hypothesis was that if two chemicals cause similar genome-wide expression changes on cultured human cells, they could potentially cause similar human responses. To test the hypothesis, we analyzed chemically induced gene expression data of cultured human cells, generated by the National Institutes of Health's Library of Integrated Network-based Cellular Signature program, and created a library of chemical genomic signatures. The signature of a chemical is a binary string of 1's representing up- or down-regulation of genes in response to chemical treatment, and 0's representing no significant change in the expression levels of the genes. We then identified drugs in the dataset that were reported to cause acute human liver and kidney injuries. To identify genes with expression changes correlating with drug-induced human liver or kidney injuries, we applied a naïve Bayesian estimator with Laplacian correction to the dataset and calculated the probability of how chemical activation of each gene correlates with human liver or kidney injuries. We then used the probabilities to predict the potential of a new chemical to cause human liver or kidney injuries based on the genomic signature of the chemical. Detailed cross-validation calculations indicated that the approach was very robust, with an area under receiver operating characteristic curve higher than 0.80 for both human liver- and kidney-toxic compounds. The results indicated that the approach may serve as an alternative to animal-based safety assessment for humans. Disclaimer: The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Army or of the US Department of Defense. This abstract has been approved for public release with unlimited distribution.

1084 Multi-Omic Analysis of Effects of Estrogen Agonists Using *In Vitro* and *In Vivo* Models Combining Genomics and Metabolomics Data

C. B. Livi[1], R. A. Fasani[1], D. R. Choudhury[1], M. Rosenberg[1], R. Chen[3], H. Li[3], A. Fornace[3], J. D. Yager[5], S. Odwin-DaCosta[5], K. Boekelheide[2], M. M. Vantangoli[2], S. Madnick[2], M. E. Andersen[4], P. D. McMullen[4], S. Pendse[4], A. Maertens[5], L. Zhao[5], T. Luechtefeld[5], A. Kleensang[5], M. Bouhifd[5], N. Garcia-Reyero[6] and T. Hartung[5]. *[1]Agilent Technologies, Santa Clara, CA; [2]Brown University, Providence, RI; [3]Georgetown University, Washington, DC; [4]Hamner Institutes for Health Sciences, Research Triangle Park, NC; [5]Johns Hopkins University, Baltimore, MD and [6]US Army Engineer Research and Development Center, Vicksburg, MS.*

There is a lot of activity around building Multi-Omic Analysis (MOA) platforms to integrate diverse data measurements from genes, proteins and metabolites. We provide features for data collection, data management, quality control, feature extraction, identification, quantification, statistical analysis and visualization as integrated single vendor workflows. Problems with study reproducibility highlight the importance of data sharing and analysis traceability. We will cover how rigorously capturing datasets and associated metadata, reproducibly selecting thresholds and creating a traceable reporting system through a hierarchical structure between data sources and the resulting figures facilitates the difficult task of documenting and reproducing analytical outputs and promoting data sharing and collaboration. Within such an environment, we show two integrated biology use cases in the area of toxicology combining gene expression and metabolomics measurements and how correlation and pathway analysis helped prioritize gene and metabolite lists for validation using a currently available commercial software platform. Both biological models address endocrine disruption including *in vitro* (human MCF7 cell line) and *in vivo* (fat head minnow tissue) models of exposure. Interestingly, based on transcriptomics, we found a core set of genes (including HSPB8, FHL2 and MYC) responsive to estradiol or Bisphenol A in both model systems. Specific results from MOA including direct comparison with previously published results indicates a common mode of action of estrogen agonists despite differing doses and profiled exposed cell types.

1085 Molecular Modeling of AhR Ligand Binding Domain Interactions with 2,3,7,8-tetrachlorodibenzo-ρ-dioxin, Indole-3-carbinol and Resveratrol

K. N. Chitrala[2], P. Nagarkatti[1,2] and M. Nagarkatti[1,2]. [1]*WJB Dorn VA Medical Center, Columbia, SC and* [2]*Pathology, Microbiology, Immunology, University of South Carolina, School of Medicine, Columbia, SC.*

Activation of Aryl hydrocarbon receptor (AhR), through ligand binding domain (LBD), leads to toxicity as well as regulation of T cell differentiation as evidenced by the effects mediated by 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD). Recent studies have shown that several dietary plant derived compounds such as Indole-3-carbinol (I3C) and resveratrol (RES) can also activate AhR. Thus, understanding how AhR LBD residues modulate the interaction with ligands would be very useful in assessing the toxicity of dioxins as well as delineating how dietary AhR ligands play a beneficial role. To that end, in the present study, we analyzed the interactions of AhR LBD with TCDD, I3C and RES using molecular modeling approach. The amino acid sequence for AhR LBD was downloaded from Uniprot database. We generated *in silico* homology models for the ligand-binding domain of mouse AhR using Modeller 9.14. The quality of the models was assessed using Ramachandran plot. Binding site residues on the AhR LBD were predicted using a scoring function followed by molecular docking with TCDD, I3C and RES. Results from homology modeling showed that the model from mouse AhR LBD was of good quality with 99% of the residues favored and allowed regions of Ramachandran plot sharing an identity more than 90% and RMSD < 2.0 Å with the template structure. Results from the molecular docking showed that AhR LBD interacts with a binding energy of -6.18 kcal/mol for TCDD, -5.32 kcal/mol for I3C and -6.81 kcal/mol for RES. In conclusion, our molecular modeling approach revealed residues in AhR LBD that are significant for exhibiting selective modulation towards TCDD, I3C and RES (Supported by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357).

1086 The Comparative Toxicogenomics Database (CTD): Expanding Exposome and Phenotype Content to Elucidate Chemical-Disease Relationships

C. J. Mattingly[2], C. J. Grondin[2], R. Johnson[2], D. Sciaky[2], B. L. King[1], J. Wiegers[2], T. C. Wiegers[2] and A. P. Davis[2]. [1]*Mount Desert Island Biological Laboratory, Salisbury Cove, ME and* [2]*Biological Sciences, NCSU, Raleigh, NC.*

The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) is a public resource that aims to advance understanding about mechanisms by which environmental exposures affect human health. Core CTD provides manually curated chemical-gene interactions and chemical-disease and gene-disease relationships. These data are integrated with molecular information and analytical tools to help generate, interpret and refine hypotheses relating to chemically influenced diseases, pathways targeted by exposures, and extrapolation from model systems. To further environmental health research and complement emerging exposome initiatives, we launched new curation modules for a) exposure science and b) chemical-phenotype data. The exposure module consists of curated information within the context of four primary exposure concepts: stressor, receptor, event, and outcome. To date over 1,700 manuscripts have been reviewed and curated data comprise approximately 53,000 measurements for 621 chemical and 71 gene markers, 245 diseases, and 146 biological processes. Integration of these data adds "real world" context to CTD while centralizing and providing broader biological context for exposure data. The chemical-phenotype module expands our disease curation by describing how chemicals influence molecular, cellular, physiological, and organismal traits that are not considered disease endpoints. These data should advance: a) understanding of environmental disease progression via pre-disease phenotypes, b) identification of potential biomarkers of exposure, c) the capacity to conduct and interpret studies across species and experimental systems. Our presentation will provide examples of these new data modules and how their integration in CTD augments the overall research capacity of this resource.

1087 ToxReporter: Viewing the Genome Through the Eyes of a Toxicologist

M. Gosink. *Investigative Toxicology, Pfizer Inc., Groton, CT.*

It is becoming standard practice within the pharmaceutical industry to evaluate new gene targets for their potential safety risks very early in the drug discovery process. Such an evaluation can save both time and money by allowing priorities to be made among otherwise equally valid targets and also highlighting potential derisking activities that can be performed concurrently with early efficacy studies. These up-front target safety evaluations require time-consuming searches of wide-ranging databases from literature reports to genetic mutations to gene annotations. Each of these sources needs to be evaluated in terms of the types of risks in order that the appropriate derisking activities can be scheduled. ToxReporter is a PERL-based web-application which utilizes a MySQL database that can streamline this process by categorizing public and proprietary domain-derived information into predefined classes of safety according to a customizable lexicon. Users can view gene information that is "red-flagged" according to the safety issue under investigation. For example, NOS2 (nitric oxide synthase 2, inducible) has a role in many diverse body processes. When examining cardiovascular issues, ToxReporter flags links to related PubMed articles spanning 171 cardiac topics ranging from "Aneurysm" to "Ventricular Remodeling" while links to articles topics related to the 130 immune-related topics remain hidden. Other types of information are similarly selectively displayed or hidden. ToxReporter also uses a scoring system based on these red-flags to rank all targets for each safety issue and to display their scores as an easily interpretable "Tox-At-A-Glance" chart. While ToxReporter was originally developed to display safety information, it is flexible enough that it could be adapted to display disease information as well.

1088 Anticancer Activity of Natural DNA Methyltransferase Inhibitors

W. Maldonado-Rojas[2], M. Olivero-Acosta[2] and E. Melendez[1]. [1]*Department of Chemistry, University of Puerto Rico, Mayagüez, Puerto Rico and* [2]*School of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia.*

The human DNA methyltranferases (DNMTs) are emerging molecular targets in epigenetic therapy. The overexpression of these DNMTs has been detected in several cancer types, including colon, breast, prostate, liver and skin, and in leukemia. Therefore finding DNA methyltransferase inhibitors (DNMTis) is a pivotal priority for cancer treatment. Virtual screening protocols showed that gambogic acid, phloridzin and digoxin have the potential to interact with DNMTs. In this work those compounds were evaluated as antineoplastic agents in human colon adenocarcinoma (HT-29) and breast adenocarcinoma (MCF-7) cells utilizing the MTT assay after 72 h exposure. The results showed that gambogic acid was most effective toward HT-29 (IC50 = 2.8 ± 0.02 nM) compared with digoxin (IC50 = 90 ± 2 nM) and phloridzin (IC50 > 450 μM). However, in MCF-7 cells, digoxin presented a higher cytotoxic activity (IC50 = 191 ± 10 nM) followed by gambogic acid (IC50= 6.02 ± 0.11 μM). Phloridzin did not elicit cytotoxic activity against MCF-7 or HT-29 cells. These three natural products also interact with human serum albumin, according to fluorescence quenching, circular dichroism, and UV-Vis spectroscopy; as well as to molecular modeling data, adding valuable information on pharmacokinetic properties for these compounds. Results presented here suggest gambogic acid and digoxin are potential anticancer drugs that may serve as scaffolds for designing new and more potent DNMTis suitable to fight against cancer (WM-R is sponsored by Colciencias, Colombia, 528-2011).

1089 Prenatal Bisphenol A Exposure Induces Changes in Global and Individual Transposon DNA Methylation in Both Mice and Humans

C. Faulk[1], J. Kim[2], M. Sartor[2] and D. Dolinoy[2]. [1]*Animal Science, University of Minnesota, Minneapolis, MN and* [2]*Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Bisphenol A (BPA) is a pervasive endocrine disruptor that affects gene expression through epigenetic reprogramming. Here we examine the impact of perinatal exposure to BPA on transposon DNA methylation in liver from mice and humans. To assay DNA methylation across the entire genome, including repetitive DNA, we developed a novel pipeline using next-generation sequencing. Human fetal livers with measured BPA levels from 35.4 to 96.8 ng/g (high), 3.5 to 5.8 ng/g (low), and <0.83 ng/g (non-detect) were used for DNA extraction. After enzymatic enrichment for high GC and methyl-CpG content, samples were sequenced via Illumina Hi-seq. Similarly, liver DNA from 3 week mice gestationally exposed to 50 mg, 50 μg, or 0 BPA/kg diet were assessed via Illumina GAIIx.

In human uniquely mapped regions, BPA levels were positively associated with methylation in CpG islands, and negatively associated with methylation in CpG shores, shelves, and repetitive regions. The main advantage of our pipeline is the detection of differential DNA methylation in repetitive regions. In humans, transposon classes: LINE, LTR, DNA element, and Satellites exhibited non-monotonic responses, with reduced methylation in low BPA samples. These shifts were mirrored in repeat families: LINE1, ERVLs, and ERVL-MaLRs, and TcMar-Tigger. In contrast, mice lacked exposure-dependent DNA methylation differences by class or family. Interestingly, at the subfamily level in humans, 15 subfamilies were distinct, all medium to young aged Alus. Mice had only a single subfamily with differential methylation, a type of major satellite. Finally, we found 1258 (human) and 23 (mouse) transposon insertions responding to BPA. Most importantly, we recapitulated the discovery of one previously known labile transposon, the Cabp(IAP) element, which was found proximal to our top hit in mice. Since only a handful of environmentally labile transposons have been discovered in animals, and none in human, ongoing work will confirm these candidates and determine whether they are true metastable epialleles.

PS 1090 Mining the Tox21 Literature with SWIFT-Review

B. E. Howard[2], J. Phillips[2], K. Miller[2], K. Thayer[1] and R. Shah[2]. *[1]National Institute of Environmental Health Sciences, Research Triangle Park, NC and [2]Sciome, LLC, Research Triangle Park, NC.*

In pursuit of the Tox21 initiative, approximately 8,000 potentially toxic chemicals are being studied as part of the broader effort by the EPA and FDA to develop and test high-throughput screening assays and methodologies. Recent research has demonstrated the usefulness of text-mining and natural language processing techniques for extracting valuable information about the likely biological impact of chemicals from latent information and relationships hidden in the scientific literature. We have developed the SWIFT-Review text mining software which can be used to identify, quantify and characterize the information density for chemicals in the Tox21 library and to prepare "scoping overviews" of the literature associated with a given chemical or set of chemicals. Given that chemical nomenclature is poorly standardized, we have built into our software a large database of synonyms that can be used to identify alternative names for Tox21 and other chemicals, with each Tox21 chemical having, on average, approximately 20 synonyms. More than 5 million PubMed citations were found to mention at least one Tox21 chemical in the title, abstract or MeSH terminology and roughly half of the 8,186 distinct Tox21 chemicals were found to have at least one associated PubMed article. Using SWIFT-Review it is possible to quickly screen the literature for co-mentions of a given Tox21 chemical and various other relevant concepts including health outcomes, environmental exposure, pharmacological actions, PubMed MeSH terms, etc. In addition, users can also build topic models for specific chemicals, cluster chemicals according to their similarities in "literature space" and use machine learning to rank order citations for further screening based on various user-specified criteria. By providing a data-driven approach to chemo-centric literature review, SWIFT-Review will facilitate a deeper contextual understanding of the science of toxicological chemicals.

PS 1091 A Bioinformatics Strategy to Enhance DILI Prediction by Integrating Diverse Predictive Models

K. McEuen[1,2], L. Wu[2], S. Thakkar[2], W. Tong[2] and M. Chen[2]. *[1]The University of Arkansas at Little Rock and the University of Arkansas for Medical Sciences, Little Rock, AR and [2]Division of Bioinformatics and Biostatistics, National Center for Toxicological Research, Jefferson, AR.* Sponsor: M. Chen.

Drug-induced liver injury (DILI), though rare, can result in severe clinical outcome such as acute liver failure and even death. Despite rigorous toxicity testing in the preclinical phase, DILI is still frequently encountered in clinical trials and has been a major cause of drug failure and market withdrawal. *In silico* modeling has demonstrated its utility in identifying the DILI risk associated with a tested compoundcompounds undergoing development before it reaches clinical trialsin drug discovery t and helping to o avoid the the potential late-stage disasters. Current research suggests that DILI is a multifaceted disease and predictive models derived from a single data type may not adequately account for all aspects of DILI and therefore limit their utility. Using an integrated approach to combine models with diverse data types may improve prediction accuracy [Chen et al., Biomarkers in Medicine, 2014]. In this study, we applied a simple voting strategy and a decision tree algorithm to integrate results from four diverse DILI predictive models, including the "Rule-of-Two" [Chen et al., Hepatology, 2013], the DILI prediction system [Liu et al., PLoS Computational Biology, 2011], a DILI predictive QSAR model [Chen et al., Toxicological Science, 2013], and an assay based consensus model [Wu et al., unpublished]. The prediction accuracies of these four individual models ranged from 51% to 72%, as estimated from the dataset of 218 drugs annotated for DILI risk based on the FDA-approved drug labeling. After investigating all possible model combinations, the highest prediction accuracy (76.1%) achieved by simple voting was derived from integrating the "Rule-of-Two," the DILI prediction system, and the assay based consensus model. The decision tree algorithm produced similar results, achieving an accuracy of 75.7% by applying the assay based consensus model to the first node followed by the "Rule-of-Two." Our study highlighted the importance of including diverse data and algorithms to improve DILI predictive models considering the complex DILI mechanisms involved. Acknowledgments: This work was funded by the Oak Ridge Institute for Science and Education

PS 1092 Integrative Analyses of MiRNA and Proteomics Identify Potential Biological Pathways Associated with Onset of Pulmonary Fibrosis in the Bleomycin Rat Model

S. Fukunaga[1,2], A. Kakehashi[1], K. Sumida[2], M. Kushida[2], H. Asano[2], M. Gi[1] and H. Wanibuchi[1]. *[1]Department of Molecular Pathology, Osaka City University Graduate School of Medicine, Osaka, Japan and [2]Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan.* Sponsor: T. Yamada.

To determine miRNAs and their predicted target proteins regulatory networks which are potentially involved in onset of pulmonary fibrosis in the bleomycin rat model, we conducted integrative miRNA microarray and iTRAQ-coupled LC-MS/MS proteomics analyses, and evaluated the significance of altered biological functions and pathways. We observed that alterations of miRNAs and proteins are associated with the early phase of bleomycin-induced pulmonary fibrosis, and identified potential target pairs by using Ingenuity Pathway Analysis. Using the data set of these alterations, it was demonstrated that those miRNAs, in association with their predicted target proteins, are potentially involved in canonical pathways reflective of initial epithelial injury and fibrogenic processes, and biofunctions related to induction of cellular development, movement, growth, and proliferation. Prediction of activated functions suggested that lung cells acquire proliferative, migratory, and invasive capabilities, and resistance to cell death especially in the very early phase of bleomycin-induced pulmonary fibrosis. The present study will provide new insights for understanding the molecular pathogenesis of idiopathic pulmonary fibrosis.

PS 1093 Mining and Visualization of Compound- and Target-Toxicity Relationships from Historical Toxicology Study Reports Using IBM Watson

R. J. Brennan[4], Z. Jayyosi[1], A. Amberg[2], D. Aldous[4], W. S. Spangler[5], I. R. Stanoi[5], S. M. Glissmann[5], Y. Chen[5] and J. Connelly[4]. *[1]DSAR, Sanofi, Framingham, MA; [2]DSAR, Sanofi, Frankfurt, Germany; [3]DSAR, Sanofi, Waltham, MA; [4]LGCR, Sanofi, Waltham, MA and [5]Watson Innovations, IBM, San Jose, CA.* Sponsor: R. Brennan.

Sanofi partnered with IBM Watson on a pilot project to investigate the ability of Watson to accurately extract and structure safety information from historical toxicology study reports, and to integrate those data with orthogonal information such as target activity data. Iterative training of a set of highly complex text annotation algorithms enabled the accurate, automated extraction of detailed experimental design from ~250 4-week rat toxicology study reports warehoused in PDF format. Data extracted included species, strain, sex, study group composition, test article, route of administration, dose levels, and dosing schedule. Significant test article-related clinical pathology and histopathological findings were also extracted using standardized lexica, and accurately associated with study groups and effect levels (where available). The extracted safety data were linked to compound-target affinity data from an SAR database, and a flexible and an intuitive search and visualization interface developed to allow the retrieval of historical data for safety outcomes by compound name/ID, target organ, specific outcome, target class, and other criteria. Innovative visualizations allowed the identification of related or coincidental safety findings, such as a frequent co-occurrence of liver necrosis with compounds causing hepatocyte hypertrophy, and of target relationships to safety outcomes, such as a link between liver hypertrophy and PPAR agonist activity. This proof of concept project demonstrates the potential of the approach for the rapid and accurate extraction of safety data from unstructured sources, and its integration with diverse, related data types for comparative or mechanistic investigation, and new insights.

1094 Predicting Potential Protein Targets for Chemical Toxins Using Molecular Similarity Search, *In Silico* Docking and *In Vitro* Validation

J. M. Gearhart[2], J. S. Frey[1], C. A. Mauzy[4] and Y. Chushak[1]. [1]*The Henry M. Jackson Foundation For Military Medicine, Wright-Patterson AFB, OH;* [2]*Biological Modeling Group, The Henry M. Jackson Foundation For Military Medicine, Wright Patterson AFB, OH;* [3]*Biological Modeling Group, The Henry M. Jackson Foundation For Military Medicine, Wright-Patterson AFB, OH and* [4]*Molecular Bioeffects Branch, Bioeffects Division, 711th Human Performance Wing, Wright-Patterson AFB, OH.*

The adverse outcome pathway (AOP) framework starts with the molecular initiating event that describes the interaction of a chemical with its macromolecular targets. Although significant amounts of information about the biological activities of toxins and toxin-target interactions are already collected using experimental methods and stored in a variety of public databases, a lot of specific information remains missing. Computational methods can be used to fill up this data gap and to provide hypotheses for future experiments. We have developed an approach for predicting the protein targets of chemical toxins using a molecular similarity search of toxin-target information collected in the Toxin and Toxin-Target Database (www.t3db.ca). The developed method identified new targets for toluene which could be used to predict potential cellular toxicities and to validate the approach with *in vitro* laboratory studies. With this new method we obtained 124 potential targets for toluene from a molecular similarity search and the results were further analyzed using *in silico* molecular docking methods. The binding of toluene to two proteins, hemoglobin and serum albumin, was validated by the measurement of binding using microscale thermophoresis. These results demonstrate the applicability of this exploratory *in silico* toxicity tool, based on a molecular similarity search and protein-ligand docking for identification of potential targets for chemical toxins.

1095 Real-Time Cell Viability Profiling of Tox21 10K Compounds

J.-H. Hsieh[2], R. Huang[3], R. Tice[1], R. Paules[1], M. Xia[3] and S. Auerbach[1]. [1]*DNTP, NIH/NIEHS, Durham, NC;* [2]*NIEHS, Kelly Government Solutions, Durham, NC and* [3]*NIH, NCATS, Rockville, MD.*

Chemicals with different cytotoxic mechanisms may present distinct cellular and temporal profiles of cytotoxicity. The real-time cytotoxicity of the Tox21 10K library was measured in HEK293 & HepG2 cells at 15 concentrations (generally 6 nM to 92 µM) using two different multiplexed assay readouts measured at 0, 8, 16, 24, 32, & 40 hours of exposure. Cell viability was quantified using the Promega RealTime-Glo MT Cell Viability Assay (glo) and the Promega CellTox Green Cytotoxicity Assay (flor); the former measures the reducing potential of cells and thus metabolism while the latter measures cellular membrane integrity. The point-of-departure (POD) at each time point was calculated and chemicals were considered active if their POD was less than the highest tested concentration. Across the different time points the assays detected 424-2253 (HEK293 glo), 560-808 (HepG2 glo), 159-1603 (HEK293 flor) and 221-1860 (HepG2 flor) actives. The number of actives increased with duration of exposure in each assay and stabilized at 16 hours of exposure for the glo assay and 32 hours for the flor assays. POD correlation analysis between assays using different cell lines suggested that the HEK293 glo assay had the most distinct response pattern among four and the HEK293 flor assay and HepG2 flor assay are similar to each other. A self-organizing map algorithm was used to cluster the compounds based on PODs. The largest cluster of active compounds (n > 700) contained mostly compounds that are active starting at 8 hours of exposure in both cell lines and assays. Chemical annotation enrichment of the clusters suggested that patterns of response have some degree of correspondence to pharmacological or toxicological class. For example, one cluster was enriched with statins (e.g., lovastatin) and another cluster was enriched for chemicals that interfere with the androgen receptor (AR) signaling (e.g., hydroflutamide and pesticides, such as propanil). Characterization of the statin and AR antagonism activity indicated that clustering of these compounds was driven by selective activity in the HepG2 flor assay and the HEK293 glo assay, respectively. In order to obtain greater differentiation of the cytotoxicity mechanism classes using this approach, more cell lines and time course sampling is needed.

1096 Human Physiologically-Based Pharmacokinetic Models Based on Quantitative Property-Property and Structure-Property Relationships

S. Chebekoue and K. Krishnan. *École de santé Publique, Université de Montréal, Montréal, QC, Canada.*

New generation of toxicological tests and assessment strategies will require human toxicokinetic data or models, which are not available for most chemicals. The aim of this study was to predict the human area under the venous blood concentration-time curve (AUC) for a subset of volatile organic compounds (VOCs) on the sole basis of their molecular structure information (CH3, CH2, CH, C, C=C, H, Br, Cl, F, Aromatic ring, Hydrogen on the aromatic ring). First, using quantitative structure-property relationships (QSPRs) for vegetable oil:air, water:air and protein:air partition coefficients (Poa, Pwa, Ppa, respectively) along with blood lipid and water content information, the blood:air partition coefficient (Pba) was predicted. Second, the intrinsic clearance (CLint) was predicted from a peer-reviewed Quantitative property-property relationship (QPPR) based on n-octanol:water partition coefficient (Pow), Pwa and ionisation potential. Finally, the integration of these QSPRs and QPPR with physiological data into a human PBPK model consisting of four compartments (liver, fat, richly and poorly perfused tissues) allowed the prediction of the 24-hour-AUC (AUC24) following eight hours exposure to 1 ppm of each of the 36 VOCs studied. The Pba predictions were on average within a factor of 2.3 ± 2.7 of the experimental data. The AUC24 values obtained with this model were within the range of predicted values when considering either no or maximal hepatic metabolism for 29 of the 36 VOCs, with smaller deviations from the expected range observed for the remaining VOCs (carbon tetrachloride, 1,1-dichloroethylene, cis-1,2-dichloroethylene, ethylene, methyl chloride, propylene and vinyl chloride). Overall, the integrated modeling approach developed in this study constitutes a pragmatic screening-level tool for predicting toxicokinetics of data-poor organic chemicals.

1097 An Automated Method to Assess and Document the Validity of *In Silico* Models

J. C. Madden[2], M. T. Cronin[2], F. Pognan[1] and C. M. Ellison[2]. [1]*Biochemical & Cellular Toxicology, Discovery Investigative Safety, Novartis Pharma AG, CH-4002 Basel, Switzerland and* [2]*School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom.*

In silico models are increasingly being employed to fill data gaps within a wide range of industries. Their appeal arises from ethical and legislative necessity to reduce animal testing; many models have been developed to predict a wide range of properties, including toxicity. However, their increased use has not been matched by increased acceptance by regulatory authorities. This is due, in part, to a lack of confidence in the validity, reliability and robustness of *in silico* models. The IMI eTOX project (www.etoxproject.eu) has been at the forefront of developing *in silico* models, for use in the pharmaceutical industry, alongside methods that can be used to assess the validity of these models. Within eTOX a protocol for model verification has been developed which is informed by the OECD principles for the validation of *in silico* models and the philosophy of good modelling practice. Verification explicitly considers factors such as training and test data (including structural accuracy), the modelling algorithm used, summary statistics, mechanistic information and applicability domain. This model verification process has now been developed into a computational application that can be distributed as a KNIME workflow. It enables automated verification of *in silico* models and produces a summary model verification report. This automated process has the potential to enable model developers and users from a wide range of industries to assess the validity of *in silico* models. Here we will present an outline of the model verification process, and details of how this has been captured in a tool for automating model verification. It is anticipated that application of this process will improve confidence in *in silico* predictions, increasing their use and acceptance across a range of disciplines. Acknowledgement: This work has been funded in part by the eTOX project, grant agreement number 115002 under the Innovative Medicines Initiative Joint Undertaking (IMI-JU).

1098 QSAR Modeling to Predict Androgen Receptor Pathway Activity

Q. Zang[3], D. Allen[3], W. Casey[1], R. Judson[2] and N. Kleinstreuer[3]. *[1]NIEHS/NICEATM, Durham, NC; [2]US EPA/ORD/NCCT, Durham, NC and [3]NIEHS/NICEATM, ILS, Inc., Durham, NC.*

The Tox21 and ToxCast programs have tested ~1800 chemicals in a broad panel of *in vitro* high-throughput screening (HTS) assays. Nine HTS assays that map to the androgen receptor (AR) pathway were integrated into a computational model to identify substances with potential androgenic/anti-androgenic activity. Here, we used the HTS dataset, the associated computational model output, and machine learning methods to develop quantitative structure-activity relationship (QSAR) models to predict AR antagonism. Although the HTS data predicted that most ToxCast chemicals would be inactive against the pathway, 225 were predicted to have some antagonist activity. QSAR classification models were trained on these data and built to predict AR antagonist activity using linear discriminant analysis, random forest, and support vector machines. Potential model variables included molecular descriptors from QikProp and MOE and structural fingerprints. Recursive feature elimination was used to extract structural features most relevant to AR activity. A training set of 1243 ToxCast chemicals with AR assay data was used to derive and optimize the binary classification models. A test set of 612 ToxCast chemicals with AR assay data was used to validate the performance of each model. Performance was assessed by overall accuracy, sensitivity, specificity and geometric mean. In addition to binary classification, the models predicted potency of the active compounds using multiple linear regression and partial least squares regression with variables selected by genetic algorithm. The best classification model was used to make predictions for a broader chemical universe, predicting that 20.6% (6475/31428) of these chemicals may have AR antagonist activity. This initial result is certainly an overestimate that is confounded by very weak activity or cytotoxicity, and further refinement of the model should improve specificity. This work does not reflect EPA policy. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No.HHSN273201500010C.

1099 QSAR-Based Prediction of Ames Mutagenicity for Regulatory Submissions

V. K. Gombar, M. S. Lawless and R. D. Clark. *Simulations Plus, Lancaster, CA.* Sponsor: S. Ferguson.

For impurities below the ICH qualification thresholds, and lacking data for classification with respect to mutagenic and carcinogenic potential, the regulatory bodies of the European Union, Japan, and USA accept their genotoxicity evaluation by (Q)SAR approaches. Regulators recommended using two approaches: "One methodology should be expert rule-based and the second methodology should be statistical-based." Here we present statistical-based QSAR models to predict outcomes of the bacterial reverse mutation assay for *E. coli* and individual strains of *S. typhimurium*, both with and without rat liver S9 metabolic activation. Each of the QSAR models, developed using information-rich structure quantifiers, is an ensemble of 33 artificial neural networks (ANNs) optimized for maximum Youden index. When tested with external test sets, the specificity of the models ranges between 82.5% and 93.0% and sensitivity between 77.3% and 100%. After running a focused subset of 2270 compounds from the World Drug Index through these models, we have developed rules to assess Mutagenicity Risk associated with a given compound. For the subset of the Hansen's benchmark test set that was not included in the training sets of our models, it was observed that 93.3% of the compounds assigned a Mutagenicity Risk of 4 by these rules were in fact mutagens. These QSAR models and rules can be accessed from the ADMET Predictor™ software v7.2 and larer.

1100 TCDD-Elicited Reorganization of Hepatic Metabolism in Support of Profibrotic Processes

T. R. Zacharewski[3], K. A. Fader[3], D. A. Ammendolia[3], P. Dornbos[3], K. Kumagai[3], J. R. Harkema[3], S. Y. Lunt[3] and R. Nault[3]. *[1]Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI; [2]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI and [3]Pathology & Diagnostic Investigation, Michigan State University, East Lansing, MI.*

We have previously shown that in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), central carbon, glutaminolysis and serine/folate metabolism are reprogrammed to support NADPH production and ROS defenses. To further investigate the underlying metabolic responses associated with TCDD-elicited fibrosis, complementary RNA-Seq, ChIP-Seq, and 28d metabolomic (urine, serum and hepatic extract) analyses with serum marker assessments were obtained from female C57BL/6 mice gavaged every 4d over a 28d or 96d period. Histological assessments revealed dose-dependent increases in hepatic fat accumulation, inflammation, and collagen deposition. TCDD increased glucose tolerance at 63d and 96d with a concomitant 3.0-fold decrease in hepatic glycogen. In serum, TCDD reduced adiponectin 2.3-fold, and induced Fgf21 >10-fold, while insulin and leptin were unchanged. Serum proinflammatory and profibrotic interleukins-1B, -2, -4, -6, and -10, as well as TNFα and IFNγ, exhibited dose-dependent induction. RNA-Seq identified the differential expression of genes involved in fibrosis such as Adams, Adamts, collagens, and Timps with a 7.7-fold increase in TGFβ2 suggesting stellate cell activation, and conflicting evidence for epithelial to mesenchymal transition (EMT). Integration of these data indicate reprogramming of glycogen, ascorbic acid, and proline metabolism consistent with collagen deposition. Collectively, these results indicate that in addition to reprogramming of NADPH production, glycogen, ascorbic acid, and proline metabolism are also reorganized to support hepatic collagen deposition in response to TCDD. Funded by Superfund Research Program P42ES04911 (TZ), Integrative Training in Pharmacological Sciences Grant 5T32GM092715 (RN) and Canadian Institutes of Health Research Doctoral Foreign Study Award DFS-140386 (KAF).

1101 Generation of Computationally Predicted Adverse Outcome Pathway Networks Through Integration of Publicly Available *In Vivo*, *In Vitro*, Phenotype, and Biological Pathway Data

N. O. Oki[2], S. M. Bell[2], R.-L. Wang[1], M. Nelms[2] and S. W. Edwards[2]. *[1]Ecological Exposure Research Division, US EPA, Cincinnati, OH and [2]Integrated Systems Toxicology Division, US EPA, Research Triangle Park, NC.*

The Adverse Outcome Pathway (AOP) framework is becoming a widely used tool for organizing and summarizing the mechanistic information connecting molecular perturbations by environmental stressors with adverse ecological and human health effects. However, the conventional process for assembly of these AOPs is time and resource intensive and has been a rate limiting step in their development. To accelerate the process, we developed computationally predicted AOPs (cpAOPs) by association mining of data from publicly available databases. A cpAOP network of ~21,000 associations was established among 105 phenotypes from TG-Gates rat liver data, 994 REACTOME pathways, 688 High-throughput (HT) assays from ToxCast, and 194 chemicals. A second network of 128,536 associations was generated by connecting 255 genes representing ToxCast biological targets to 4,980 diseases from the Comparative Toxicogenomics Database (CTD) using either HT screening activity from ToxCast for 286 chemicals or CTD gene expression changes in response to 2,330 chemicals. Each network was separately evaluated by manual extraction of disease-specific cpAOPs and comparison with expert curation of the relevant literature. Both networks were then merged with ~130,000 publicly available phenotypes from PhenomicDB covering several model species, to create a single resource for automated cpAOP extraction and prioritization via the use of probability and weighting metrics. This provides a more comprehensive hypothetical AOP list than is possible by expert evaluation alone. A review of the most likely putative AOPs given an outcome of interest can then be performed by experts. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.

1102 Investigating Key Event-Based Points of Departure for Adverse Oxidative Events in Human Bronchial Epithelial Cells Exposed to Zinc: A Systems Biology Approach

J. M. Currier[1], W.-Y. Cheng[1], R. Conolly[2] and B. Chorley[2]. *[1]ORISE/US EPA, Research Triangle Park, NC and [2]US EPA, Research Triangle Park, NC.*

Determining key event-based points of departure that distinguish between adaptive and adverse cellular processes is critical to assist in predicting adverse health outcomes associated with environmental exposures. Utilizing a systems biology approach, we characterized cellular responses of the tracheobronchial airway to a model oxidant exposure, zinc (Zn2+). A pharmacokinetic model for normal human bronchial epithelial (BEAS-2B) cells exposed to Zn2+ and pyrithione, an ionophore facilitating cellular uptake, was developed to predict the saturation of intracellular Zn2+ sequestration necessary for eliciting adverse oxidative effects leading to apoptosis. Computational simulations predicted that 2-10 μM Zn2+/pyrithione would increase free intracellular Zn2+. Concentration- and time-dependent cytotoxicity with exposures of 3–10 μM Zn2+/pyrithione was confirmed *in vitro* using BEAS-2B cells.

Zn2+ exposure conditions eliciting adaptive and apoptotic responses were then characterized by assessment of NRF2 and p53 pathway activation. Differences in global gene expression under normal, adaptive, and apoptotic Zn2+ exposure conditions were assessed to delineate underlying molecular mechanisms. After 4 h, 154 genes were differentially expressed (p<0.01) between the adaptive and apoptotic Zn2+ concentrations. Further bioinformatic analyses identified 67 genes associated with p53 and/or NRF2 signaling pathways. Using existing gene expression and ChIP-seq databases, 30 of the candidate genes were selected for qPCR analysis. Overall, this work suggests that the switch between adaptation and apoptosis in our model begins to occur at exposures of approximately 3 µM Zn2+ and as early as 4 h after exposure. Future work will identify key event-based points of departure for adverse (versus simply adaptive) events to support expansion of the Zn pharmacokinetic model to assist prediction of adverse outcomes. This abstract does not necessarily reflect the policy of the US EPA.

1103 Pyruvate Kinase Isoform Switching and Hepatic Metabolic Reprogramming by the Environmental Contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

R. Nault[2], K. A. Fader[2], M. P. Kirby[2], S. Ahmed[5], J. Matthews[4,5], A. D. Jones[2], S. Y. Lunt[2] and T. R. Zacharewski[2]. *[1]Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI; [2]Chemistry, Michigan State University, East Lansing, MI; [3]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI; [4]Nutrition, University of Oslo, Oslo, Norway and [5]Pharmacology & Toxicology, University of Toronto, Toronto, ON, Canada.*

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) elicits dose-dependent hepatotoxicity that includes fat accumulation, inflammation, and fibrosis that may progress to hepatocellular carcinoma. To further investigate these effects, RNA-Seq data was integrated with computationally identified putative dioxin response elements (pDREs), and complementary targeted metabolomic and aryl hydrocarbon receptor (AhR) ChIP-Seq data from female C57BL/6 mice gavaged every 4 days for 28 days. Data integration using CytoKEGG with manual curation identified alterations in central carbon and amino acid metabolism. More specifically, TCDD increased pyruvate kinase isoform M2 (PKM2) gene and protein expression. PKM2 has lower catalytic activity resulting in decreased glycolytic flux and the accumulation of upstream intermediates that were redirected to the pentose phosphate pathway (PPP) and serine/folate biosynthesis, two important NADPH producing pathways. In addition, the GAC:KGA glutaminase (GLS1) protein isoform ratio was increased, consistent with increases in glutaminolysis which serves an anaplerotic role for the TCA cycle and compensates for the reduced glycolytic flux. Collectively, gene expression, protein, and metabolite changes were indicative of increased NADPH production in support of cytochrome P450 activity and ROS defenses. This AhR-mediated metabolic reprogramming is similar to the Warburg effect that represents a novel advantageous defense mechanism to increase anti-oxidant capacity in normal differentiated hepatocytes. Funded by Superfund Research Program P42ES04911 (TZ), Integrative Training in Pharmacological Sciences Grant 5T32GM092715 (RN) and Canadian Institutes of Health Research Doctoral Foreign Study Award DFS-140386 (KAF).

1104 Multiscale Systems Modeling of Male Reproductive Tract Defects: From Genes to Populations

M. C. Leung[2,3], R. M. Spencer[1] and T. B. Knudsen[3]. *[1]Lockheed Martin, Research Triangle Park, NC; [2]Oak Ridge Institute for Science and Education, Oak Ridge, TN and [3]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC.*

The reproductive tract is a complex, integrated organ system with diverse embryology and unique sensitivity to prenatal environmental exposures that disrupt morphoregulatory processes and endocrine signaling. US EPA's *in vitro* high-throughput screening (HTS) database (ToxCastDB) was used to profile the bioactivity of 54 chemicals with male developmental consequences across ~800 molecular and cellular features. The *in vitro* bioactivity on molecular targets could be condensed into 156 gene annotations in a bipartite network. These results highlighted the role of estrogen and androgen signaling pathways in male reproductive tract development, and importantly, broadened the list of molecular targets to include GPCRs, cytochrome-P450s, vascular remodeling proteins, and retinoic acid signaling. A multicellular agent-based model was used to simulate the complex interactions between morphoregulatory, endocrine, and environmental influences during genital tubercle (GT) development. Spatially dynamic signals (e.g., SHH, FGF10, and androgen) were implemented in the model to address differential adhesion, cell motility, proliferation, and apoptosis. Urethral tube closure was an emergent feature of the model that was linked to gender-specific rates of ventral mesenchymal proliferation and urethral plate endodermal apoptosis, both under control of androgen signaling. A systemic parameter sweep was used to examine the sensitivity of crosstalk between genetic deficiency and environmental disruption pathways. The *in silico* impact of subtle variations in SHH and FGF10 activity (simulating individual variability) with respect to dose-dependent androgen receptor insufficiency was modeled in a simulated population. Hypospadias could occur at 70% SHH and 70% FGF10 sufficiency with merely a 5% reduction in AR sufficiency. In conclusion, multiscale systems modeling provides a means to simulate population-level responses to reveal critical thresholds in teratogenesis for complex interactions between genetic (e.g., FGF10 polymorphism), environmental (e.g., androgen receptor disruption), and lifestyle (e.g., cholesterol deficiency for SHH) factors. (Disclaimer: this abstract does not reflect US EPA policy).

1105 Microphysiological Systems (MPS) to Identify Organ-Organ Interactions in Toxicology: Hepatic Metabolism Enhances Nephrotoxicity of Aristolochic Acid

S.-Y. Chang[2], E. Weber[2], E. J. Kelly[2], D. L. Eaton[2] and T. Neumann[1]. *[1]Nortis, Inc., Seattle, WA and [2]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA; [3]Pharmaceutics, University of Washington, Seattle, WA.*

To develop improved *in vitro/ex vivo* models for identifying potentially nephrotoxicity chemicals, we initially developed a kidney-on-a-chip platform using human proximal tubule epithelial cells (PTECs), and a liver-on-a-chip using rat or human hepatocytes grown in microfluidic devices (MPS) developed by Nortis, Inc. To test the hypothesis that hepatic clearance of a nephrotoxic chemical might have significant importance in determining ultimate kidney toxicity, we utilized Aristolochic acid (AA), a well-known nephrotoxin and carcinogen, that undergoes extensive hepatic metabolism. The specific activation and detoxification pathways for AA in liver and/or kidney are not fully understood. Prior studies in our laboratory demonstrated that induction of hepatic CYP3A4 increased the formation of a non-toxic AA metabolite (AA-Ia). We therefore hypothesized that passing AA through a MPS with liver cells prior to introducing the output flow into a kidney MPS would decrease nephrotoxicity. Interestingly, using LIVE/DEAD® vital stain data to quantify the extent of nephrotoxicity after 24 hour treatment, we found that hepatocyte exposure substantially increased toxicity in PTECs compared to direct exposure of the PTECs to AA. IC50s were 5.35 µM (liveràkidney) v.s. 66.52 µM (direct kidney) in rat PTEC cells and 17.84 µM (liveràkidney) v.s. 77.52 µM (direct kidney) in human PTEC cells (p< 0.01), indicating that hepatic metabolism apparently contributes more to activation of AA than to detoxification. We also detected the increased kidney injury marker (KIM-1) in effluents of liveràkidney MPS group after 25 µM AA treatment for 24 hours (2.3 fold increased compared to the vehicle control, p< 0.01). These results demonstrate that *in vitro/ex vivo* bioactivation of AA in the liver impacts its nephrotoxicity, and support the hypothesis that *in vitro/ex vivo* integrated MPS culture systems can be used to identify toxicologically relevant organ-organ interactions that may occur *in vivo*. Our results might explain the historical question that AA toxicity specifically occurs in kidneys. Our integrated MPS model is a new approach to investigate organ-organ interaction *in vitro/ex vivo*.

1106 Systems Toxicology Assessment of a Candidate Modified Risk Tobacco Product Compared to a Combustible Cigarette

I. Gonzalez Suarez[1], F. Martin[1], D. Marescotti[1], E. Guedj[1], S. Acali[1], S. Johne[1], R. Dulize[1], K. Baumer[1], D. Peric[1], D. Goerdetier[1], S. Frentzel[1], N. V. Ivanov[1], C. Mathis[1], A. W. Hayes[2], M. C. Peitsch[1] and J. Hoeng[1]. *[1]Philip Morris International, Neuchatel, Switzerland and [2]Spherix Consulting, Rockville, MD.*

Cigarette smoke increases the risk for respiratory and other diseases. Cessation is the most effective approach to minimize the risk for smoking-related diseases. However, for those unwilling to quit smoking, modified risk tobacco products (MRTP) may be useful to lower disease burden. The tobacco heating system (THS2.2) is a candidate MRTP where tobacco is heated rather than burned. THS2.2 is composed of an electronic holder where the tobacco stick is inserted and heated by an electronically-controlled heating blade. Heating the tobacco generates an aerosol mainly composed of water and glycerol that contains lower levels of harmful and potentially harmful constituents (HPHC). Primary human bronchial epithelial cells were used to investigate the biological impact of THS2.2 compared to the 3R4F combustible cigarette.

Multiple toxicity endpoints were measured via real-time cellular analysis and high-content screening. The study was complemented by gene expression analysis, followed by a computational approach to identify and quantify perturbed molecular pathways. Chemical characterization of THS2.2 aerosol showed similar nicotine levels and reduced levels of 53 HPHCs compared to 3R4F smoke. Cells were exposed to 3 different smoke/aerosol fractions: an aqueous extract generated by bubbling mainstream 3R4F smoke or THS2.2 aerosol through PBS, total particulate matter (TPM) and gas-vapor phase (the substance that passes through the filter during TPM collection). Exposure to 3R4F smoke fractions caused a dose-dependent response in most toxicity endpoints and a significant level of perturbation in multiple biological pathways, particularly in those related to cellular stress. By contrast, exposure to THS2.2 aerosol fractions had an overall lower biological impact. No cellular toxicity was observed at similar doses and up to 15-fold increased THS2.2 concentrations were necessary to elicit a level of response similar to that of 3R4F fractions. Moreover, the level of biological network perturbation was also significantly reduced following THS2.2 exposure compared to 3R4F. In summary, our systems toxicology study demonstrated reduced effects of aerosol fractions from the candidate MRTP, THS2.2, compared to the combustible cigarette 3R4F.

PS 1107 A Multiplatform Metabolic Phenotyping Approach Integrated with Pathway Mapping to Identify Biochemical Differences Between Healthy Smokers and Non-Smokers

M. R. Kaluarachichi[1], C. L. Boulange[1], I. Garcia-Perez[1], J. C. Lindon[1] and E. Minet[2]. *[1]Bio-Incubator, Metabometrix Ltd, London, United Kingdom and [2]R&D Centre, British American Tobacco, Southampton, United Kingdom.* Sponsor: M. Gaca.

A biological system can be simplified to a network of genes, proteins, metabolites interacting with each other in equilibrium. A toxicological stress can lead to a temporary or permanent perturbation of this network. Thus if a detailed signature of biological perturbations caused by an exposure event can be obtained and mapped to biological pathways it becomes possible to model the causal relationship between exposure and adverse events leading to diseases. In the context of risk assessment, creating a comprehensive map of the perturbed biological functions caused by tobacco smoking could be useful for comparison against novel tobacco/nicotine products. The objective of this study was to perform a comprehensive multiplatform comparison (NMR, UPLC-MS RP+/-, UPLC-MS HILIC+/-) of the serum metabolome/lipidome of smokers (n=56) and non-smokers (n=57). Multivariate statistical modelling and univariate statistic allowing for confounder variables was performed. From this a panel of 110 metabolic biomarkers was produced that distinguish the two classes. 88 were tentatively identified of which 62 varied by 10% or more between the two groups at pFDR<0.01. Some of the strongest changes were observed in smokers for L-tryptophan (-1.6x), hypoxanthine (+2.2x), and 1-acylglycerophosphocholine (-1.7x). The identified metabolites were used as input for metabolic pathway enrichment analysis using IPA Ingenuity® and the Reactome database to predict adverse biological events. Lipid peroxidation, glutathione metabolism, and inflammatory response were predicted. Those are known adverse biological events in smokers indicating that combining a metabolomics approach with knowledge-base mapping can give mechanistic insights into disease development. A similar approach can be applied to next generation tobacco/nicotine products for risk assessment.

PS 1108 Classifying Carcinogens Using Weighted Gene Co-expression Network Analysis (WGCNA)

B. M. Paisley[1,5], J. J. Sutherland[3,5], R. A. Jolly[2,5], T. K. Baker[1,5] and J. L. Stevens[4,5]. *[1]Investigative Toxicology, Eli Lilly and Company, Indianapolis, IN; [2]Risk Assessment/Global REACH, Eli Lilly and Company, Indianapolis, IN; [3]Tailored Therapeutics, Eli Lilly and Company, Indianapolis, IN; [4]Toxicology, Eli Lilly and Company, Indianapolis, IN and [5]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN.*

Gene expression data has been used to classify genotoxic and non-genotoxic carcinogens using supervised approaches, e.g. gene signatures, *in vivo* and *in vitro*. We derived 415 co-regulated gene networks (modules) encompassing 8014 rat liver genes from DrugMatrix using an unsupervised WGCNA approach. To assess the utility of modules, 9071 expressed liver genes, and 1840 canonical pathway or gene ontology terms to classify carcinogens we categorized compounds in the TG-GATES database as: 1) DNA reactive carcinogens (Ames positive); 2) non-DNA reactive genotoxic carcinogens (e.g. clastogens); 3) non-DNA reactive and non-genotoxic carcinogens; and, 4) non-carcinogens. We then used liver gene expression from 1, 4 and 8 days (d) of treatment to identified genes, pathways and modules with q-value <0.01 in categorizing carcinogens from non-carcinogens (1,2,3v4), or DNA-reactive from other carcinogens (1v2,3). Compared to 4d or 8d, the 1d data provided the best discrimination for 1vs2,3 (4-50x more significant features for all methods), and for 1,2,3v4 (2-12x) classification tests. Compared to the top 10 genes, pathways and modules were 71% vs. 65% as effective for the 1v2,3 comparison, and 71% vs 93% for the 1,2,3v4 comparison, respectively. However, module performance exceeded that of genes and pathways when comparisons were normalized to the top-ranked 2.5% of genes (277), pathways (46) or modules (10) at each time point. Further, of the top-ranked modules on any day, 22% (4) are top-ranked across all 3 days, compared to 2.5% for pathways (3) and 5.5% (30) for genes; 18 of the top 30 genes mapped to 2 of the top 4 modules. We also used the rat liver classifiers for all 3 methods to classify carcinogens from 33 mouse liver treatments, the top 10 modules, pathways and genes have comparable effect sizes of 0.51, 0.42 and 0.48 (Cohen's d statistic). In summary, carcinogen classification using modules compares favorably with conventional gene-based approaches with lower time-dependence and similar translation across species.

PS 1109 Application of the SILAC Method to Identify Protein Adducts of PCB Quinones

M. Li[3], Y. Li[3], M. Pope[3], L. Robertson[3] and G. Ludewig[3]. *[1]Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA; [2]Occupational and Environmental Health, University of Iowa, Iowa City, IA and [3]Proteomics Core Facility, University of Iowa, Iowa City, IA.*

The observation of high levels of airborne PCBs in old school buildings raises concerns of inhalation exposure and toxicity of these PCBs. Lower chlorinated PCBs (LC-PCBs), major components of airborne PCBs, are subject to biotranformation. *In vitro* and *in vivo* studies revealed that reactive metabolites of LC-PCBs form covalent adducts with DNA and proteins. Profiling the target proteins of reactive PCB metabolites could help to better understand the toxic mechanism of LC-PCBs, but no such study was found. We attempted to identify target proteins of PCB3 (4-monochlorobiphenyl) metabolites. We used MALDI-TOF and LC-QTOF to identify adducts of the PCB3 quinones (PCB3-Q) on proteins *in vitro*. A previous kinetic study found that PCB quinones react instantaneously with sulfur nucleophiles, suggesting cysteine residues on proteins as the most susceptible binding sites. Our experiments with cell lysate did not show any PCB3-Q adducted through cysteines but rather through lysine residues. Incubating trypsin pre-digested albumin with PCB3-Q, cysteine adducts were found which decreased significantly during incubation in pH 7 buffer at 37 °C, but the 'un-adducted' peptides did not reappear. The 'instability' of these adducts may be caused by further redox reactions of quinones and crosslinking. We then used the stable isotope labeling by amino acids in cell culture (SILAC) method which compares amounts of heavy and light proteins serving as sample and control, respectively, to indirectly identify the target proteins based on the disappearance of one of the twin peaks. Using this approach, target proteins were identified in labeled cell lysates incubated with PCB3-pQ. These results show that multiple steps involved in proteomic methods will lead to a decrease of cysteine adducts, making it impossible to directly identify target proteins by proteomic methods (false negatives). However, the SILAC method will provide indirect evidence of adduction that can be used to identify target proteins. (Funded by NIEHS P42 ES013661)

PS 1110 Translational Biomarkers of Acetaminophen-Induced Hepatotoxicity in Urine Samples

L. K. Schnackenberg[3], J. Sun[3], J. Greenhaw[3], S. Bhattacharyya[1], P. Gill[2], W. F. Salminen[5], D. L. Mendrick[4], L. P. James[1] and R. D. Beger[3]. *[1]Arkansas Children's Hospital Research Institute, Little Rock, AR; [2]Arkansas Children's Research Institute, Little Rock, AR; [3]National Center for Toxicological Research, US FDA, Jefferson, AR; [4]National Center for Toxicological Research, US FDA, Silver Spring, MD and [5]ProNatural Brands LLC, Sarasota, FL.*

Acetaminophen (APAP) is a commonly used over-the-counter drug analgesic and large doses can lead to acute liver failure and death. APAP accounts for approximately half of the cases of ALF in the United States with over half of those due to an unintentional overdose. More specific translational biomarkers of APAP overdose are needed since the current approach relies on accurate timing of overdose and ALT levels, which are not necessarily specific to liver injury. Previous non-clinical metabolomics studies in urine from male Sprague Dawley rats identified potential biomarkers of APAP-induced hepatotoxicity from the tricarboxcylic acid cycle, ketone metabolism, and the tryptophan pathway. In this extended clinical study, urine samples were collected from healthy

(N = 6, median age 14.08 years) and overdose patients (N = 11, median age 13.91 years) as part of an IRB-approved multicenter study of APAP toxicity in children. The median level of ALT in the overdose patients was 1572 IU/L, about 100 fold higher than that of the control group. Levels of APAP protein adducts were significantly higher, 1.02 nmols/L, in the overdose group compared to 0.01 nmols/L for controls. NMR- and UPLC/QTof-MS-based metabolomics methods were employed to evaluate the urine samples and identified compounds from pathways previously noted to be altered in the non-clinical APAP studies including citrate, isocitrate, 3-hydroxybutyrate, indoxyl sulfate, tryptophan, and xanthurenic acid. The results indicate a metabolomics approach may enable the discovery of specific biomarkers of drug toxicity in animals and humans improving drug development and clinical management of patients.

1111 Stably Expressed Genes in Rat Liver - Sex Differences Involved in Liver Function and Drug Metabolism

K. Wang, V. Vijay and J. Fuscoe. *US FDA, Jefferson, AR.*

Genes expressed in the liver at a relatively constant level during the lifespan may be involved in crucial hepatic functions whose disruption may result in toxic responses. To identify such genes, we examined gene expression profiles of liver tissues from 4 male and 4 female untreated F344 rats at 2, 6, 21, and 104 weeks of age. The expression variability of each of the 19343 genes was calculated as the maximum expression level divided by the minimum expression level, which ranged from 1.18 to >109 fold. Sex-specific Stably Expressed Genes (SEGs) were defined as those genes whose expression varied by ≤ 2-fold across the 16 female or male liver samples. 2153 SEGs were found in females and 1813 SEGs in males. Among them, 564 SEGs were unique to males, 904 SEGs were unique to females, and 1249 genes were stably expressed in both sexes. SEGs common to both sexes were significantly (FDR<0.05) enriched in pathways of oxidative phosphorylation, liver disease, protein processing, RNA transport, and the TCA cycle. For the male-specific SEGs, no consistent pathways were identified. However, the female-specific SEGs were associated with pathways involving ribosomes, the lysosome, amino acids degradation, and fatty acid degradation. In addition, the sex-specific expression stability of the genes encoding cytochrome P450 (CYP) enzymes was examined. The genes encoding 5 CYP enzymes (Cyp2a2, Cyp2d1, Cyp2d5, Cyp3a23/3a1 and Cyp4a3) were stably expressed only in females, while only 2 (Cyp2t1 and Cyp4v3) were exclusively stable in males. In conclusion, sex difference in SEGs suggested that some crucial hepatic functions (e.g., synthesis and breakdown of proteins) may be more stable in the liver of female than in male rats and that this difference may result in differential susceptibility to drug-induced liver injury. The sex differences in stability in the expression of genes encoding CYP enzymes during the lifespan may influence the metabolism and toxic responses of relevant drugs.

1112 Comparison of Clinical Pathology Data from Pen/Group Housed and Individually Housed Cynomolgus Monkeys

C. Voyer and A. Provencher. *Charles River, Sherbrooke, QC, Canada.* Sponsor: M. Vezina.

In alignment with the 3Rs, group housed non-human primates (NHPs) have become more common for nonclinical studies compared to the more traditional individually housing paradigm. Our objective was to evaluate potential differences related to housing conditions on standard clinical pathology parameters by comparing baseline data from pen/group- housed animals versus individually housed animals. Clinical pathology data from 93 male and 92 female pen/group-housed NHPs was compared with data from 94 male and 64 female individually-housed NHPs. All animals were experimentally naïve, healthy, adult (2 to 6 years old) cynomolgus monkeys, fed with the same diet and maintained in comparable room environmental conditions. Blood samples were collected from animals fasted overnight (individually-housed only). Clinical pathology evaluations were performed with the following analyzers: Siemens Advia® 120 (hematology), Stago STAR 4® (coagulation) and Roche P800® (biochemistry). Levene's test was used to assess the homogeneity of group variances parametric assumption at the 5% significance level. Datasets were compared using a two-sided t-test (Levene's test or Wilcoxon Rank-Sum test (when Levene not significant)) and all significant pairwise comparisons reported at the 0.1, 1, and 5% significance levels. Statistically significant changes were seen in hematology, coagulation and biochemistry. Taking into account expected biological variability for the species and assay methodology, noteworthy changes were: 71–73% decreased neutrophil, 37–46% increased lymphocyte and 85–62% increased eosinophil counts, possibly indicative of lower stress levels in the pen/group housed animals. Clinical chemistry changes consisted of 20–27% increased urea, 86–89% decreased phosphorus, 5–5% decreased total protein and 8–11% decreased globulin. As comparison to baseline and to concurrent controls data determines potential experimental compound effect on clinical pathology, the observed differences were considered not to influence overall outcome and interpretation for pen/group-housed NHPs in nonclinical studies.

1113 A Mechanism-Based Hepatotoxicity Simulation Model to Capture Dose-Dependent Cell Death Dynamics in Response to the Drugs Amiodarone and Valproate

A. Crudu[2], R. Note[2], A. Riu[2], B. Fromenty[1], G. Ouedraogo[2], O. Perin[2], S. Jager[2], C. Gomes[2], S. Teissier[2], I. Suzuki[2], K. Fujita[3], Y. Matsuoka[3], S. Ghosh[3] and H. Kitano[3]. [1]*INSERM UMR991, Rennes, France;* [2]*L'Oréal, Aulnay-sous-Bois, France and* [3]*The System Biology Institute, Tokyo, Japan.* Sponsor: E. Dufour.

The ability to predict systemic toxicity is becoming increasingly important for the Cosmetic Industry, particularly in the context of the ban on animal testing. While Adverse Outcome Pathways (AOPs) elucidate the knowledge-base for mechanisms of toxicity, dynamics simulation models need to be considered to have a better understanding of the time course of the toxic response. The screening of legacy data showed that the liver was the organ potentially affected by cosmetic ingredients upon systemic exposure (Vinken et al, Arch Toxicol 2012). Assuming that mitochondrial dysfunction is a major mechanism whereby hepatotoxicity occurs, we initiated a collaborative project on the development of a model for estimating the dose that defines the transition from an adaptive to a toxic response through this mechanism. Hence, we built a mechanistic mitochondrial map describing hepatic steatosis and necrosis through perturbation of the energetic balance. Based on this map, we developed a simulation model, which captures the interactions through ordinary differential equations (ODEs). Further, hybrid optimization techniques were applied to estimate model parameters to fit the observed experimental data in HepaRG cell line under chemical perturbations by amiodarone and valproate. The best-fitted model successfully reproduced the observed 12 days-time course of loss of ATP concentration and mitochondrial membrane potential loss, as well as cell death in response to escalating doses of the two chemicals. Our study demonstrates the capability of mechanism-based simulation modeling for enhancing the scope of *in silico* study of xenobiotic dynamics of toxicity. In an effort to fine tune our model, the next step will consist of performing a series of additional experiments designed to cover both the compensatory and toxic events describing the proposed AOP.

1114 Woodsmoke Exposure Alters Serum Composition That Induces Inflammatory Gene Expression and Impairs *Ex Vivo* Wound Healing

M. Campen[2], L. Heine[2], J. Liu[1], J. Brower[1], G. Herbert[2], B. Sanchez[2], K. Zychowski[2], L. Topper[2], M. Doyle-Eisele[1] and J. McDonald[1]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM and* [2]*Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

Woodsmoke inhalation continues to be a major environmental exposure worldwide, with much of the world dependent on biomass burning for heat and cooking. With increasing lifespans, globally, the role of chronic exposure to biomass combustion in driving chronic diseases such as atherosclerosis requires greater study. Earlier studies of a single 6 h exposure to woodsmoke in healthy mice found that the serum from those mice could increase endothelial cell inflammatory gene expression and reduce vasorelaxation in a panel of *ex vivo* assays. In the present study, we examined the impact of subchronic (6h/d x 50d) exposure to hardwood (Oak) smoke at 450 µg PM/m3 on changes in serum composition that drives endothelial inflammatory and growth impairment outcomes. Serum from exposed ApoE-/- mice was added to the media of endothelial cells in culture in a dilute (2.5%-30%) manner. In a wound-healing assay, primary murine cerebrovascular endothelial cells incubated with serum from woodsmoke-exposed mice exhibited significant deficits in regrowth compared to serum from control (filtered air-exposed) mice. Of note, the regrowth curves normalized at the 30% serum concentration but showed a maximal difference between groups at the 2.5% concentration, suggesting that exposure to woodsmoke leads to a loss of some growth factor, rather than induction of some anti-growth molecule. In parallel assays, endothelial cells incubated with 5% serum from exposed or control mice for 4h demonstrated a significant increase in VCAM-1 mRNA expression. Aortas from woodsmoke-exposed mice showed minimal changes in inflammatory gene transcripts,

relative to filtered air controls. These findings suggest that circulating inflammatory bioactivity due to acute woodsmoke inhalation may subside in subchronic exposures, but would healing impairments may persist. Future studies will need to assess whether such changes in serum bioactivity confer premature aging or stiffness to vascular beds.

1115 The Effect of Antibiotic Treatment on Microbiome Metabolites Observed in Rat Blood

C. Behr[3], B. van Ravenzwaay[3], H. G. Kamp[3], E. Fabian[3], G. Krennrich[3], W. Mellert[3], E. Peter[1], V. Straus[3] and T. Walk[1]. *[1]Metanomics GmbH, Berlin, Germany; [2]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany and [3]Quantum Chemistry, BASF SE, Ludwigshafen am Rhein, Germany.*

Microbiome and host interactions play an important role in well-being. The microbiome is known to produce several metabolites (e.g. short chain fatty acids, amino acids, vitamins and hormones) which are important for the host's physiology. Antibiotics have a profound effect on the micro-organism composition of the microbiome and hence on the mammalian-microbiome co-metabolism. The consequences, however, on the functionality of the microbiome (defined as the production of metabolites absorbed by the host) is not well known. We have employed a metabolomics approach to investigate these effects. To this purpose broad-spectrum antibiotics belonging to the class of aminoglycosides (streptomycin sulfate, neomycin sulfate, gentamicin sulfate), fluoroquinolones (moxifloxacin hydrochloride, levofloxacin) and tetracyclines (doxycycline monohydrate, tetracycline hydrochloride) were administered for 28 days to male rats with blood metabolic profiling after 7, 14 and 28 days. For each class of antibiotics specific and selective metabolome patterns could be established. To distinguish between metabolite changes caused by systemic toxicity of the antibiotics (e.g. liver and kidney toxicity) and microbiome related changes, the metabolites identified in the metabolome pattern were compared to a list of metabolites known to be produced by the gastro-intestinal micro-organisms. Beside changes mainly concerning amino acids and carbohydrates, hippuric acid and indole-3-acetic acid were identified as key metabolites being affected by antibiotic treatment. The following gut metabolites were found to be unique for each class: Aminoglycosides: indole-3-propionic acid; Fluorquinolones: taurine; Tetracyclines: 3-indoxylsulfate, uracil, allantoin. The results of these investigations suggest that blood based metabolic profiling (metabolomics) could be a suitable tool to investigate the effect of antibiotics on the functionality of the microbiome and to obtain detailed insight in the mammalian-microbiome co-metabolism of endogenous metabolites.

1116 Metabolic Network of Estrogen Metabolism in Human Mammary Gland: Validation and Influence of Soy Isoflavones

K. Schmalbach[3], A. Cecil[3], R. Hauptstein[3], S. T. Soukup[4], D. Pemp[3], C. Kleider[3], L. N. Geppert[6], C. Köllmann[6], P. Eckert[1], I. Neshkova[5], T. Dandekar[3], S. Kulling[4], H. Esch[3] and L. Lehmann[3]. *[1]Group Practice for Plastic Surgery and Hand Surgery Wuerzburg, Wuerzburg, Germany; [2]Chair of Food Chemistry, University of Wuerzburg, Wuerzburg, Germany; [3]Department of Bioinformatics, University of Wuerzburg, Wuerzburg, Germany; [4]Department of Safety and Quality of Fruit and Vegetables, Max Rubner-Institute, Karlsruhe, Germany; [5]Department of Surgery II, University Hospital of Wuerzburg, Wuerzburg, Germany and [6]Faculty of Statistics, TU Dortmund, Dortmund, Germany.*

A contributing factor to breast cancer development is the female sex hormone 17β-estradiol (E2), which can be converted to reactive metabolites known to form adducts with the DNA. Intake of soy isoflavones (IF, for example soy-based food) is known to reduce the risk of breast cancer in Asian women, whereas in Western women no association has been observed. Hence, the influence of IF on the activation of E2 to genotoxic metabolites in female mammary gland tissue was investigated using computational based metabolic network modelling. Based on tissue levels of E2 and estrone quantified by means of GC-MS/MS and on transcript levels of 22 E2 metabolizing enzymes determined by quantitative TaqMan PCR, the metabolic flux of DNA adducts of 45 mammary gland tissues, of women on soy-rich or soy-deficient diet or taking an IF-rich extract, were modelled. In addition to 159 reactions of the E2 metabolism the network model also contained 66 reactions of energy metabolism, using published microarray data from human platelets as constraints. Furthermore, tissue levels of genistein and daidzein, their conjugates and their bacterial metabolites were quantified by means of UHPLC-MS/MS. Then, linear regression models with metabolic flux of estrogen DNA adducts as dependent variable and age, body mass index, number of pregnancies, smoking habits, intake of estrogen active drugs and IF tissue levels (all as explanatory variables) were examined. 17 reasonable combinations of tissue levels of IF aglyca, conjugates and biotransformation products were used as explanatory variables in 17 individual models. No IF-related explanatory variable significantly influenced the metabolic flux of DNA adducts, whereas both age (negative relationship) and intake of E2-releasing drugs (positive correlation) had a significant influence. DFG, Le-1329/10-1.

1117 Using Connectivity Mapping Approach to Evaluate Modes of Action of Large Set of Chemicals in MCF7 Cells

Y. Shan, J. M. Naciff, X. Wang, X. Wei, L. A. Reinsalu, R. C. Schinaman and G. P. Daston. *Procter & Gamble, Mason, OH.*

Based on our previous work of using the L1000 gene expression assay and connectivity mapping (cMAP) approach to define chemical modes of action (MOAs), we examined gene expression profiles in MCF7 cells after exposure to 108 additional chemicals using same approach. Among the 108 chemicals, 48 are from Sigma LOPAC®1280 library, which contains marketed drugs and relevant compound structures to provide predictable activity profiles against a wide range of MOAs; the other 60 chemicals are from the ToxCast Phase 2 list. Each chemical was tested at 3 different concentrations after 6h exposure. Experiments were designed to ensure treatment balance between the 3 replicates and across batches. Statistical analysis showed that 58 chemicals have at least one concentration with 10 or more significantly expressed genes (FDR<0.1); 49 chemicals have at least one concentration with 1 or 9 significantly expressed genes; only 1 chemical didn't have any significantly expressed genes at any concentrations. cMAP analysis revealed that gene signatures generated from the most expressed genes were consistent with known MOAs of chemicals already in the cMAP database. For instance, gene signatures of reserpine and ellipticine showed cMAP database connections in a concentration-dependent manner with reserpine and ellipticine, respectively; and their related classes are also highly ranked. Cytarabine, a DNA synthesis inhibitor is not in the cMAP database, but connected strongly with chemicals that inhibit DNA synthesis that are in the database. 4-nonylphenol, 4-octylphenol and daidzein, all showed strong concentration-dependent, estrogenic connections and there are other examples where the cMAP approach were successful in identifying MOAs. The results further confirmed that use of cMAP is appropriate to evaluate the MOAs of unknown chemicals. Work is planned to evaluate the gene expression profiles of several other cell lines exposed to the same group of chemicals using the cMAP approach.

1118 Intestine-Liver Interactions in TCDD-Elicited Steatohepatitis

K. A. Fader[2], R. Nault[2], D. A. Ammendolia[2], T. A. Lydic[2], R. B. Crawford[2], N. E. Kaminski[2] and T. R. Zacharewski[2]. *[1]Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI and [2]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI; [3]Pharmacology & Toxicology, Michigan State University, East Lansing, MI; [4]Physiology, Michigan State University, East Lansing, MI.*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) elicits dose-dependent hepatic fat accumulation that progresses to steatohepatitis with fibrosis. To investigate the role of the intestine, we examined the dose-dependent effects of TCDD on jejunal gene expression in C57BL/6 mice treated by oral gavage every 4 days for 28 days. Agilent 4x44K microarray analysis of the jejunal epithelium identified 439 differentially expressed genes (DEGs; |fold change| ≥ 1.5, P1(t) ≥ 0.999) associated with lipolysis, fatty acid/cholesterol absorption and transport, the Kennedy pathway, and retinol metabolism, consistent with increased hepatic fat accumulation. Moreover, major histocompatibility complex (MHC) class II genes were repressed, coincident with decreased macrophage and dendritic cell levels in the lamina propria, suggesting migration of antigen presenting cells out of the intestine. Reciprocal increases in hepatic MHC class II expression and macrophage infiltration lead us to speculate that immune cells may migrate from the intestine to the liver. TCDD also increased gastroduodenal and colonic para-cellular permeability, and whole gut transit time, suggesting gut leakiness, decreased gut motility, and bacterial overgrowth. Accordingly, serum levels of cholic acid and bacterial-derived deoxycholic acid were increased following repeated TCDD treatment. These results demonstrate TCDD-elicited changes are consistent with hepatic lipid accumulation, macrophage migration, inflammation, and the progression to steatohepatitis. Funded by Superfund Research Program P42ES04911 (TZ), Integrative Training in the Pharmacological Sciences Award 5T32GM092715 (RN), and Canadian Institutes of Health Research Doctoral Foreign Study Award DFS-140386 (KAF).

1119 A Multi-scale Network Perspective on the Aryl Hydrocarbon Receptor Toxicity Pathway

N. Shilpa, M. E. Andersen and S. Bhattacharya. *Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Several recent reports and vision statements on the future of chemical safety assessment have emphasized the need for a computational network analysis and modeling-based approach to the investigation of toxicity pathways and adverse toxic outcome pathways. Multiple gene expression and transcription factor-gene interaction datasets are available in the literature for relevant environmentally-perturbed pathways like the estrogen receptor, peroxisome proliferator-activated receptors alpha and gamma, and the aryl hydrocarbon receptor (AhR). These genome-wide datasets can serve as tools for identification and reconstruction of the biological networks underlying adverse response. Here we present an analysis of the transcriptional regulatory network underlying the AhR toxicity pathway in mouse liver. We used a combination of gene expression and genome-wide transcription factor binding data to derive the regulatory network for TCDD-induced activation of AhR in the mouse. Superposing gene expression time course data on the network revealed the regulatory logic and sequential activation among the nodes in the network. Analysis of the AhR network with Kohonen self-organizing maps and subspace clustering algorithms showed significant overlap between gene co-regulation and gene co-expression, while Lasso regression identified the transcription factors most predictive of gene expression patterns. Gene ontology analysis revealed differential activation of several groups of biological processes, including metabolism, immune response and kinase activation. This network mapping and modeling procedure shows the manner in which a perturbation cascades through multiple cellular signaling paths, and can be used to support a quantitative framework for safety assessment of environmental chemicals acting through core toxicity pathways.

1120 Development of a Predictive Systems Toxicology Platform to Enhance Novel Molecule Design

K. Johnson[2], M. LeBaron[2], S. Sriram[1], K. Yang[1], J. Raymond[1], B. Veeramani[1], R. Rasoulpour[1] and N. Elango[1]. [1]*Dow Agrosciences, Indianapolis, IN and* [2]*The Dow Chemical Company, Midland, MI.*

A key aspect of novel molecule development is assessment of acute to chronic mammalian toxicity and risk to human health. Utilizing only classical approaches for toxicity assessment can result in substantial animal use, time and resources, which could be wasted if long-term toxicity studies identify hazards of significant concern. To this end, the opportunity presented by developing robust predictive toxicity platforms for novel molecule design is far-reaching in impact. In this project, a bioinformatics platform was created using high-content biological datasets to predict a wide-range of toxicity effects. To develop the first generation of this platform, RNA-seq transcriptome data from rat liver were obtained after 28 day exposure to fourteen molecules. The chosen candidates bridged to public datasets (clofibrate and phenobarbital), were predicted to have similar effects in liver (three glycol ethers), or were novel molecules with a wide range of toxicities. These RNA-seq data were combined with publicly available microarray transcriptome rat liver profiles to identify bioinformatics processes that would most accurately bin molecules by liver toxicity type. Bioinformatics processes examined included unsupervised clustering, machine learning, connectivity mapping, and a novel gene network-based analysis using rat liver regulons developed using the ARACNE algorithm. Unsupervised clustering using differentially expressed genes accurately grouped RNA-seq profiles by chemical class or known liver toxicity mode of action but required a robust transcriptional response. Using connectivity mapping, the most similar TG-GATES and DrugMatrix datasets to phenobarbital and clofibrate RNA-seq were molecules with similar nuclear receptor activation potential. Gene set enrichment analysis of regulons in the gene regulatory network showed this technique could accurately bin chemicals by chemical class and mode of action. These findings demonstrate the utility of transcriptome profiling to predict rat liver toxicity. Refinement of bioinformatics processes and addition of new omics data are expected to expand the applicability domain and identify best practices for a wider array of complex toxicities.

1121 Importance of Chemical Delivery Methods for High-Throughput Screening

L. Truong, S. M. Bugel, M. T. Simonich and R. L. Tanguay. *Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

The toxicology field is faced with the challenge that there are tens of thousands of chemicals in commerce, but only a small subset of these has undergone safety assessments. These chemicals represent diverse structural classes that were developed for specific purposes (e.g., flame retardants, plasticizers, insecticides, etc.), while others are byproducts or breakdown products. The current method of using animal models has proven to be too laborious and costly. In an attempt to keep pace with this trend, high-throughput screening (HTS) assays have been developed and implemented. During an *in vivo* HTS screen, we discovered that the instrumentation and methods used to deliver test chemicals to multi-well plates containing developing zebrafish can dramatically affect the observed biological responses. The current instrumentation used for HTS testing (metal transfer/delivery pins) was prone to adsorbing chemicals on the pins. For this study a subset of chemicals, 17α-Ethinylestradiol (EE2), 17β-Estradiol (E2) and Thiram, was delivered to embryonic zebrafish using a liquid handler system, and by digital dispensing of chemicals directly into experimental chambers. Embryos were dechorionated at 4 hours post fertilization (hpf), and chemicals were added (64 nM to 64 uM) by the two chemical delivery systems. At 120 hpf, the exposed embryos were evaluated for morphological responses. We identified a remarkable concentration-response difference resulting from the different delivery technologies; embryos exposed to EE2 by the digital dispensing resulted in 100% mortality at 64 uM, while 34% mortality at 64 uM using the liquid handler. This trend was observed for E2, but not Thiram. Additional characterization was conducted by evaluating gene expression responses of vitellogenin, and aromatase in embryos exposed to 0.01–10 uM. When E2 and EE2 was delivered by digital dispensing, there was 300–700 fold induction for both genes compared to the liquid handler. Our results importantly provide a path to improve HTS reproducibility for chemical screening.

1122 Glutamine, Glutaminase and γ-Glutamyl-Transferase Activities Are Essential for Lung Tumorigenesis

D. R. Sappington[2], R. B. Penney[2], E. Siegel[2] and G. Boysen[2]. [1]*University of Arkansas for Medical Sciences, Little Rock, AR and* [2]*Environmental and Occupational Health, University of Arkansas for Medical Sciences, Little Rock, AR.*

Increased glutamine uptake has been found to drive cancer cell proliferation, making tumor cells glutamine-dependent or even addicted. Glutamine is believed to provide additional carbon and nitrogen sources for cell growth. The first step in glutamine utilization is its conversion to glutamate by glutaminase (GLS). Glutamate is a precursor for glutathione synthesis, a pathway known to be activated in tumor cells. Additionally, glutathione concentrations are known to be elevated in non-small cell lung cancer (NSCLC) and is believed to protect against oxidative stress. In contrast, glutathione's participation in the γ-glutamyl-cycle is less well studied. Here we investigated the hypothesis that glutamine drives glutathione synthesis and export, drives the γ-glutamyl-cycle, and thereby contributes to NSCLC tumor cell proliferation. Glutamine consumption and glutathione excretion were measured in H460, A549 and MRC-5 cell lines. Treatment with BPTES, a known GLS inhibitor, led to reduced cell proliferation. This was also demonstrated when γ-glutamyl-transferase (GGT) was inhibited with acivicin. GGT transfers glutamyl groups from glutathione to amino acids to facilitate the amino acid transport into the cell. These results suggest active utilization of the γ-glutamyl cycle in the tumor cells. The formation of metabolites downstream of GLS and GGT were essentially abolished at the lowest inhibitor concentrations studied. In contrast, biological endpoints, such as MTT assay response, required up to 30-fold higher inhibitor concentrations. Culturing in stable isotope-labeled glutamine demonstrated that >50% of excreted glutathione and >80% of γ-glutamyl amino acids were derived from glutamine. Together these results demonstrate that glutamine is actively utilized for synthesis and excretion of glutathione which, in turn, is utilized to promote amino acid uptake by the formation of γ-glutamyl amino acids. These findings add yet another important function to the glutamine dependence of tumor cells. In addition to providing TCA cycle intermediates, we show that glutamine promotes glutathione synthesis to increase cellular defense mechanisms, and drives the γ-glutamyl cycle to enhance amino acid uptake.

1123 Families of Polyproline Peptides in Human Butyrylcholinesterase Tetramers

H. Peng. *Eppley Institute for Cancer Research, University of Nebraska Medical Center, Omaha, NE.* Sponsor: O. Lockridge.

Butyrylcholinesterase protects the cholinergic nervous system from organophosphorus nerve agents by scavenging these toxins. BChE tetramers in human plasma consist of 4 identical subunits plus a polyproline peptide. The tetrameric quaternary configuration maintains the ability of BChE to stay in the circulation with therapeutic activity. The polyproline peptides are noncovalently bound with high affinity. They are released only when the protein is denatured. The polyproline peptides function as glue to assemble 4 subunits into a tetramer, by interacting with a series of aromatic residues located near the C-terminus. Mass spectrometry identified 116 polyproline peptides released from denatured human BChE, containing 9 to 21 consecutive proline residues and up to 7 non-proline residues typically near either end. A blast search identified only a few possible precursor proteins from human or bacteria, leaving the source of many of the polyproline peptides unidentified. RAPH1 (Ras-associated and pleckstrin homology domains -containing protein 1) protein is in the set. The findings enhanced our understanding of the assembly of human BChE.

1124 Integrating Multi-Omic Data with Apical Endpoints in a C57BL/6J Diet-Induced Obesity Mouse Model

K. P. Glover[4], J. Caverly Rae[4], Y. Dragan[4], F. Burns[4], M. Mawn[4], H. M. Jensen[4], C. C. Yde[1,4], P. Mukerji[4], L. Stenman[4] and S. Loveless[4]. *[1]Aarhus University, Aarhus, Denmark; [2]Corporate Center for Analytical Services, DuPont, Newark, DE; [3]Haskell Global Centers, DuPont, Newark, DE and [4]Nutrition and Health, DuPont, Newark, DE.*

Metabolic syndrome constitutes a cluster of conditions related to abnormalities in energy usage leading to an increased risk of cardiovascular disease and diabetes. Research indicates that a high fat/low fiber diet is a major factor in the development of metabolic syndrome, with mechanisms linked to altered intestinal permeability and changes in the intestinal microbiota. However, due to the multifactorial nature of this syndrome, more research is needed to evaluate the systems level effects of a high fat diet. C57BL/6J mice that were fed a high-fat diet (60% fat) for 18 weeks developed traits associated with metabolic syndrome such as weight gain, reduced glucose tolerance and altered serum biomarkers. To gain a systems level understanding of metabolic syndrome multiple omics endpoints were captured including: 1) RNA expression patterns in multiple tissues and intestinal sections, 2) metabolomics (both NMR and LC/MS methodology) on serum urine and liver and 3) metagenomics analysis of microbial population diversity in the feces. Gene expression changes were most pronounced in liver and adipose tissues, with alterations associated with cholesterol biosynthesis, lipid metabolism, inflammation and gut permeability. Results correlated with histopathological and clinical chemistry biomarkers associated with fat infiltration in the liver and inflammation in the adipose tissue. Differences in bile acid metabolism and the FXR pathway between ileum, jejunum and colon sections were also observed. Metabolomics analysis revealed differences in the relative abundance of over 1000 metabolites in the serum and urine. The metabolic difference was related to changes in energy, amino acid and microbial metabolism. Lastly, a significant divergence in microbial population diversity correlated with the length of time on the high-fat diet. Overall, this integrated analysis of traditional endpoints with multi-omic data provided an enhanced view of mechanistic changes associated with metabolic syndrome and high-fat diet.

1125 Redox Proteomics of Environmental Low-Dose Cadmium and Pulmonary Health

Y.-M. Go, J. Chandler, M. Orr, L. Hao and D. P. Jones. *Medicine, Emory University, Atlanta, GA.*

Lung effects of Cd from cigarette smoke and occupational sources have been extensively studied, yet low-level environmental Cd also occurs in human diet and accumulates *in vivo* due to a twenty-year biologic half-life. Our previous studies showed that environmental low dose Cd altered protein redox states resulting in stimulation of inflammatory signaling, actin cytoskeleton disruption, and fatty liver. However, little is known about low-level oral Cd on the subcellular redox proteome in lung and potential impact on pulmonary health. In the current study, we investigated effects of oral low-dose Cd on mouse lung proteome using combined methods of redox proteomics and bioinformatics. Redox proteomics was performed on lungs isolated from control C57BL/6 mice (no Cd) and mice that were exposed to Cd by drinking water (3.3 mg/L) for 16 weeks. Redox proteomics identified 548 protein peptides containing cysteine residues (peptidyl Cys) in both control (n=3) and Cd (n=3) lung samples. Of these, Cd oxidized 173 (32%) peptidyl Cys with 1.3-fold or higher than control. Mean percent oxidation of these peptidyl Cys was 20.5% and 30.5% in control and Cd, respectively, suggesting that chronic low level Cd exposure resulted in lung protein oxidation. In addition, subcellular compartment-specific analysis showed that mitochondrial proteins were most sensitive to Cd-increased oxidation in lung (25 peptidyl Cys, 1.8-fold by Cd). The results of MetaCore pathway analysis showed that Cd-oxidized proteins are associated with pulmonary diseases including cystic fibrosis, chronic obstructive pulmonary disease, lung cancer, and asthma linked to mitochondrial and cytoplasmic pathways for phosphatidylinositol-4,5-diphosphate metabolism (p=3.7e-3), linoleoyl-glycerol 3-phosphate metabolism (p=4.4e-3), carbohydrate metabolism associated with pyruvate metabolism (p=7.9e-3) and TCA metabolism (p=2.9e-3). Taken together, the results show that redox proteomics identifies subcellular compartment-specific protein oxidation in lungs of mice exposed to low-dose Cd in drinking water. Association of oxidized proteins with functional pathways of lung disease suggests utility to understand complex mechanisms of low-level environmental Cd in disease.

1126 Determining the Role of AhR in Immunoglobulin Expression Using CRISPR Cas 9

N. Panstingel and C. Sulentic. *Pharmacology and Toxicology, Wright State University, Dayton, OH.*

The Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor. AhR has been shown to mediate the toxic effects of the environmental toxin 2,3,7,8-tetrachlorododibenzo-p-dioxin (TCDD). In various animal models, TCDD inhibits the differentiation of B-cells into antibody secreting cells and the expression of immunoglobulin (Ig). Within the immunoglobulin heavy chain locus (IGH) are 3′ regulatory regions (3′RR). The TCDD-induced inhibition occurs in an AhR dependent manner that is partially regulated by the 3′RR. In humans, the effect of TCDD on the immunoglobulin heavy locus (IGH) is still unclear; however in human derived cell lines a TCDD-induced suppression of IgG has been shown. There are structural differences in the human and mouse IGH 3′RR. Within the mouse 3′RR, are four enhancers (hs3A, hs1,2, hs3B and hs4) while the human 3′RR there are only three (hs3A, hs1,2, and hs4). The hs1,2 region is also known to be polymorphic, in humans, and is associated with multiple autoimmune disorders. Because of the growing number of auto-immune disorders linked to the polymorphism within the hs1,2 region, determining the role of AhR in the IGH expression would provide valuable insight into possible health risks and aid in the development of possible treatments. To determine the role of AhR in suppression of human IGH by TCDD, CRISPR/Cas 9 genetic editing technology was utilized to disrupt the endogenous AhR expression in a CL-01 cell line isolated from a Burkitt's lymphoma. Knockdown of AhR was verified by western blot. Immunoglobulin secretion was measured using ELISA and a relative quantification of transcripts of the human IGH was measured using RT-PCR. The results obtained from ELISA show a decrease in AhR expression inhibits the overall secretion of IgG independent of TCDD exposure in shRNA constructs. From RT-PCR, it was shown that a decrease in AhR expression inhibits the germline transcripts of the g1 region of the IGH in shRNA contructs. The results obtained suggest a significant physiological role of AhR on IgG expression and that a functional AhR may be necessary for TCDD-induced inhibition of other classes of immunoglobulins. CRISPR/Cas 9 genomic editing,CYP1A1 induction and sequencing of the CRISPR modified regions will be used for further analysis of the AhR knockdown clones.

1127 BDE-153 Demonstrated Liver Toxicity in Models of Hepatic Cells in Monolayer and Tridimensional Cultures

L. C. Pereira[1], F. V. Duarte[4], A. T. I. Varela[4], A. P. Rolo[4], C. M. Palmeira[4] and D. J. Dorta[1]. *[1]Clinical, Toxicological and Bromatological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto, Brazil; [2]Departamento de Química, University of São Paulo, Ribeirão Preto, Brazil; [3]Department of Life Sciences, University of Coimbra, Coimbra, Portugal and [4]Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal.*

PBDEs are used in a wide array of polymer-containing products and, more precisely the BDE-153 congener is one of the most present in high concentrations in human samples and also found in animal wildlife. To know the mechanism of toxicity of these compounds toxicological tests are used, based on many different cellular lines culture. However, the mechanism of action of this compound is not well described. This study

characterizes the *in vitro* toxicity the BDE-153, assessed in different hepatic cell lines using monolayer (2D) and tridimensional cell culture by hang drop (3D cell culture) for more predictive data, in an attempt to elucidate the mechanism of toxicity in different cell lines. HepG2 cells were maintained in DMEM medium and plated in 12-well plate for adhesion or in 96-well hanging drop plate for 3D cell culture. Hepatocytes were isolated from healthy Wistar rats; after cannulating the portal vein, the liver was perfused with Krebs-Henseleit buffer. All cell were exposed to BDE-100 (0.1-25μM). After 24 and 48 hours of exposure to BDE-153 cell viability was assessed using 0.5% MTT. In addition, the determination of mitochondrial membrane potential (Dψ) was performed using the TMRM, and also the total protein/cell mass was evaluated using the SRB assay. Finally, we assessed the damage caused by BDE-153 in HepG2 3D-cultures using the fluorescent dyes Hoechst and Ethidium. BDE-153 has been shown to cause cellular dysfunctions by dissipating Dψ and decreasing cell viability and all cultures in monolayers. Additionally, it was observed an increase in the number of cells labeled with ethidium in HepG2 3D-culture, also indicating cell death. We concluded that the exposure to BDE-153 induces significant damage in both, immortalized and in primary cells, and demonstrated to induce cell death in 3D culture too, a new cell culture model that has been growing in recent years. This damage corroborate with the induction of mitochondrial damage data previously submitted. Supported by: Capes - Proc. PVE A018/2013; FAPESP - Proc. 2012/13123-0.

1128 Liver Transcriptomic and Metabolic Reprogramming After Exposure to Diesel Exhaust

G. Ramanathan[2], Y. Zhao[1], F. Yin[2], M. E. Rosenfeld[3], X. Yang[1] and J. A. Araujo[2]. [1]*Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, CA;* [2]*Medicine, UCLA School of Medicine, Los Angeles, CA and* [3]*Pathology and Environmental and Occupational Health Sciences, University of Washington, Los Angeles, CA.* Sponsor: M. Campen.

Background: Exposure to air pollution has been shown to associate with cardiovascular morbi-mortality, insulin resistance and type 2 diabetes. We investigated the mechanisms how air pollution induces metabolic abnormalities in the liver using a combination of ohmics approaches. Methods: Apolipoprotein E (ApoE) null mice were exposed to either diesel exhaust (DE) or filtered air (FA) for 2 weeks. For transcriptomics, RNA was extracted from livers, n=8 per group, and used for Illumina microarrays. Metabolomics analysis of the livers, n=5 per group, was performed by Metabolon Inc. Illumina data was analyzed using the Genomestudio software. *In vitro* experiments used HepG2 cells treated with a methanol extract of diesel exhaust particles (DEP) vs. media. mRNA expression levels were quantified by qPCR. Glucose and glycogen content were determined by biochemical and histological determinations. Results: Liver cholesterol and triglyceride content were significantly increased following DE exposure. Microarray analysis showed differential expression of 477 genes in the DE group compared to FA ($p< 0.05$). Pathways significantly dysregulated by DE included lipid and lipoprotein metabolism, lysosome, oxidative phosphorylation and the citric acid cycle. Metabolomics identified 118 biochemicals with significant differences in their levels between the DE and FA groups. Important pathways enriched with these metabolites included glutathione metabolism, citric acid cycle, glycogen metabolism and glycerolipid metabolism. We identified several key driver genes from the transcriptomic analysis, including PEPCK for biological validation by *in vitro* experiments. HepG2 cells treated with 100ug/ml DEP extract for 8 hours showed a marked increase in PEPCK expression suggesting enhanced gluconeogenesis. DEP treatment also resulted in depletion of glycogen content in HepG2 cells. Conclusion: Integrated analysis of liver transcriptomic and metabolomic data from mice exposed to diesel exhaust for 2 weeks indicated several alterations in metabolic pathways involved in lipid and carbohydrate metabolism, resulting in increased synthesis of triglycerides, increased gluconeogenesis and glycogenolysis.

1129 Extrapolating Salivary Acinar Cell *In Vitro* Pesticide Transport to Whole Animals Using Computational Modeling

C. Timchalk, Z. A. Carver, T. J. Weber and J. N. Smith. *Pacific Northwest National Laboratory (PNNL), Richland, WA.*

Non-invasive biomonitoring with saliva has potential to significantly advance quantitative dosimetry as an integral component of epidemiology. However, predictions of which chemical and/or metabolites are excreted into saliva at detectable concentrations remain a challenge. In order to predict salivary clearance, a combination experimental and computational approach has been developed with 3,5,6-trichloro-2-pyridinol (TCPy) (the major chemical-specific metabolite of chlorpyrifos) to predict potential salivary levels. A Transwell *in vitro* rat salivary acinar cell system was utilized, where protein levels in the basolateral (27.2 mg/mL) and apical (1.3 mg/mL) culture chambers, which represent blood and saliva compartments respectively, were altered using bovine serum albumin to more closely mimic physiological levels. TCPy was dosed to the basolateral chamber at two concentrations (250 and 2500 μM), and both chambers were sampled over time up to 24 hr. The rat salivary cell system maintained the protein gradient and tight junctions over the duration of the experiment as evidenced by consistent transepithelial electrical resistance levels. Levels of TCPy were quantified using gas chromatography-mass spectrometry, and at 24 hr, the predicted median saliva/blood concentration ratio was similar to published values measured *in vivo* (0.021 vs. 0.049). TCPy concentrations were modeled using a mechanistic computational cellular transport model. The resulting model simulations fit the data reasonably well, and fit parameters suggest that TCPy is transported across basolateral and apical cell membranes by passive diffusion. Model parameters were integrated into a physiologically based pharmacokinetic model, and reasonably predicted TCPy concentrations in rat saliva after intravenous administration of TCPy. This poster demonstrates the utility of this combination experimental and computational approach to predict chemical transport in saliva. Supported by CDC/NIOSH grant R01 OH008173.

1130 Non-Invasive Saliva Human Biomonitoring: Development of a Transwell Assay to Support Exposure Science Assessment and Health Impacts

T. J. Weber, J. N. Smith, Z. A. Carver and C. Timchalk. *Health Impacts & Exposure Science, Pacific Northwest National Laboratory, Richland, WA.*

The use of saliva as a biomonitoring matrix can significantly advance quantitative dosimetry as an integral component of epidemiology. A major limitation has been an inability to identify which chemicals are readily cleared in saliva, at levels that can be quantified. To address this limitation, we have developed a primary salivary gland serous-acinar cell model that can be used for chemical transport studies *in vitro*. Serous-acinar biomarkers detected by Western blot include alpha amylase and aquaporin 5 which are uniformly expressed in confluent cultures. Serous-acinar cells express the tight junction marker zona occludens-1 and measurements of transepithelial electrical resistance (TEER) demonstrate exceptional tight junction formation (routinely >2000 Ω/cm2) in a relatively short period of time (6-8 days) in Transwell inserts. When TEER values are > 2000 Ω/cm2, lucifer yellow transport from apical to basolateral chambers is ~ 0.1%/hr. The insecticide chlorpyrifos (CPF) and its primary metabolite trichlorpyridinol (TCPy) can be quantified in saliva at concentrations that are less than, but parallel to blood levels. Results from the Transwell assay indicate that chlorpyrifos transports by diffusion with transport rates that are linear among doses tested. Lucifer yellow passage across the epithelial barrier was clearly disproportional to chlorpyrifos transport. Primary serous-acinar cells showed a low level of CPF metabolism to TCPy, which subsequently localized to both apical and basolateral chambers, consistent with a diffusional process and lack of TCPy concentration gradient. These experiments establish the feasibility of utilizing an *in vitro* cell based uptake/clearance assay coupled with pharmacokinetic modeling as a novel chemical screening strategy to identify ideal chemical candidates for saliva biomonitoring. Future studies will begin linking the transwell assay to higher content chemical screening capabilities to increase throughput. Supported by CDC/NIOSH grant R01 OH008173.

1131 2, 4 - Dichlorophenoxyacetic Acid (2, 4-D) Transport Across an *In Vitro* Salivary Acinar Cell System: A Novel Approach to Biomonitoring

Z. A. Carver, C. Timchalk, J. N. Smith and T. J. Weber. *Health Impacts & Exposure Sci, Pacific Northwest National Laboratory, Richland, WA.*

The non-invasive use of saliva for biomonitoring has the potential to significantly advance quantitative dosimetry as an integral component of public health. A major limitation for this approach has been an inability to identify which chemicals are readily cleared in saliva, at levels that can be quantified at relevant exposure levels. 2, 4-D is a widely used herbicide and its renal clearance mechanism involves both glomerular filtration and active organic ion transport. 2, 4-D was dosed in the basolateral chamber at 2 different concentrations (10 or 100 μg/mL), and both chambers were sampled longitudinally up to 24 hr post dosing. At these doses and time-points cells were viable and maintained tight junction function based upon: transepithelial electrical resistance (TEER) and lucifer yellow permeability (LY) testing. 2, 4-D concentrations in the apical chambers increased over time, and 2, 4-D concentrations in both

chambers were equivalent within 24 hours post dosing with the high dose; however with the low dose, basolateral 2, 4-D concentrations remained higher than apical 2, 4-D concentrations (p=0.04) at 24 hr. To evaluate the potential role of active transport, a competitive substrate experiment with the OAT substrate para-aminohippuric acid (PAH), was conducted (0, 0.515, 5.15, or 51.5 µg/ml PAH + 10 µg/ml 2, 4-D) at 4 hr post-dose. Preliminary evaluations suggest that PAH did not significantly alter the concentration of 2, 4-D transported across the SGEC monolayer at 4 hours, suggesting that passive processes may predominate under these conditions. Once the salivary transport mechanism and kinetic parameters of 2, 4-D have been fully characterized, results will be incorporated into a mechanistic computational cellular transport model and used to further inform a PBPK for 2,4-D. This approach, once established, can be exploited for human biomonitoring without the need to conduct more challenging *in vivo* saliva clearance studies. Supported by CDC/NIOSH grant R01 OH008173.

1132 Navajo Birth Cohort Study: Metal Biomonitoring and Source Attribution

J. Ong[2], J. Hoover[2], C. Shuey[1], E. O'Donald[2], M. Cajero[2] and J. Lewis[2]. [1]*Southwest Research and Information Center, Albuquerque, NM and* [2]*Community Environmental Health Program, University of New Mexico, Albuquerque, NM.*

The Navajo Birth Cohort Study (NBCS) investigates the relationships among exposure to atomic bomb and Cold War Era legacy mixed metal wastes from >1100 uranium mine waste sites on Navajo Nation (NN), birth outcomes, and child development. Although the last mine were closed in 1986, communities are chronically exposed to the unremediated wastes. The study is being conducted as a cooperative agreement among CDC/ATSDR, Navajo Area IHS, Navajo Department of Health, Southwest Research and Information Center, and University of New Mexico's Community Environmental Health Program. Of the target of 1500 families, 568 mothers, 406 babies, and ~30% of fathers are currently enrolled in the study. Blood and urine samples collected at multiple time points from enrollment until baby is one year of age are analyzed for 36 metals at CDC/ATSDR/DLS/IRAT labs. Measured body burden of metals is likely due to contributions from multiple exposure pathways. Thus, to obtain a complete exposure profile, in-home dust swipes are analyzed for 24 metals, and surveys querying land-use activities, occupational and health history, and nutritional information are administered. Concentrations of uranium, manganese, mercury, antimony, tin and tungsten in NBCS participants exceed those expected relative to the US population (NHANES, 2015). To date, no predictor by itself explains these elevated levels, though correlations between blood mercury (p=0.36) and lead (p=0.05) concentrations are significantly correlated with home dust levels. Modeling of urine uranium concentrations including limited land-use predictors (local food consumption, family mining history, home heating) indicates wood and/or coal burning as a significant (p=0.04) predictor. Source attribution modeling incorporating additional exposure profile information from surveys, particularly nutritional information, is in progress for the metals for which NBCS participants exhibit unexpectedly high concentrations. Source attribution is a vital aspect of NBCS in order to identify exposure mitigation strategies and environmental clean-up prioritization.

1133 Increased Oxidative Stress Status, Cadmium, Lead and Selenium of Roadside Dispensers of Gasoline in Nigeria

O. M. Akinosun[2] and A. Sanni[1]. [1]*Chemical Pathology Department, University College Hospital, Ibadan, Nigeria and* [2]*Chemical Pathology Department, University of Ibadan, Ibadan, Nigeria.*

Gasoline, a very volatile petrochemical used for fueling automobiles and some power generating machines contains organic and inorganic constituents which when activated leads to continuous production of reactive oxygen species (ROS) and consumption of antioxidants. Work place exposure to toxic chemicals is a major public health concern worldwide and exposure to gasoline vapors has been associated with a great deal of hematological alteration, increased risk of malignancies and other chronic diseases among humans and rats. This risk is greatest in the developing countries, including Nigeria, where limited facilities to reduce over exposure to the toxic effects of the chemicals are rampant. Knowledge on the safe handling and transportation of chemicals is also very limited. This study aimed at assessing the oxidative stress status of road side gasoline dispensers in Nigeria by assaying their plasma total antioxidant status, cadmium, lead and selenium as paradigm. Materials and Methods: Markers of oxidative stress including plasma levels of total anti oxidant status (TAS), antioxidant trace metal, selenium and known free radical generating heavy metals (lead and cadmium) were compared between 90 road side dispensers of gasoline and 90 age and sex matched controls. Plasma selenium, lead and cadmium were analyzed using Atomic Absorption Spectrophotometer (AAS) and total anti oxidant status, was determined using standard colorimetric methods. Results: study shows statistically significantly higher levels of oxidative stress and heavy metals, (Pb & Cd) in road side dispensers of gasoline compared to the controls. This is an indication that road side gasoline dispensers are probably at greater risk of developing chronic diseases associated with increase oxidative stress and heavy metal toxicity. Antioxidant supplementation may be of benefit to the road side gasoline dispensers and legislation on road side gasoline dispensing should be enforced to reduce incidence of long term complications from exposure.

1134 Validation of Dried Blood Spot Method for Detection of Aflatoxin B1-Lysine Adduct in Animals and Humans

K. Xue, L. Tang and J.-S. Wang. *Environmental Health Sciences, University of Georgia, Athens, GA.*

Aflatoxin B1 (AFB1), a potent mycotoxin and group 1 human carcinogen, is a ubiquitous contaminant among groundnuts- and corn-based foods. Public health concerns has been raised for AFB1-induced growth stunting and impediments in growth and development among infant and children in many low-income developing countries. Dried blood spots (DBS), popularly used in new-born congenital disease screening, were proposed as a potential means for screening biomarkers of exposure for various environmental toxicants, due to its less invasiveness and ease of storage and shipment. We previous developed a HPLC-based experimental protocol to detect AFB1-Lysine adduct in DBS samples. This study aimed to validate the analytical protocol and examine the correlation between AFB1 exposure and levels of AFB1-Lysine adduct in DBS samples in animals and human samples. F344 rats were treated with single or repeated-dose AFB1 and DBS cards were prepared from the whole blood. Serum was also prepared from the whole blood to serve as comparison purpose between two methods in detection of AFB1-Lysine adduct. DBS cards prepared from human whole blood were spiked with known levels of AFB1-Lysine adduct and human serum samples from Kenya and Uganda were used for validation study. A significant dose and time effects of adduct levels were found in DBS cards treated with single- and repeated-dose AFB1. Pearson correlations comparing AFB1-Lysine adduct levels in DBS and serum samples were 0.997 for the single-dose study, 0.998 for the repeated-dose study, and 0.843 for spiked human samples. Using Bland-Altman plot, the log difference between the results of two sample types were found to be within limit of agreement approximately 95% of the time for both human and animal samples. Our results showed that AFB1-Lysine adduct levels in DBS cards and serum samples from animals and from spiked human samples are comparable, and the DBS technique and analytical protocol is ready to move to field study aimed to assess AFB1 exposure in infant and children populations.

1135 Prenatal Exposure to Pyrethroid Insecticides Metabolites Measured in Umbilical Cord Blood Serum

M. Wren[1], M. Liu[1], A. Vetrano[4], B. Buckley[1], J. R. Richardson[2] and S. L. Shalat[3]. [1]*Department of Environmental and Occupational Medicine, Rutgers, The State University of New Jersey, Piscataway, NJ;* [2]*Department of Pharmacy Practice, College of Pharmacy, Northeastern Ohio Medical School, Rootstown, OH;* [3]*Divison of Environmental Health, School of Public Health, Georgia State University, Atlanta, GA and* [4]*Neonatology, Robert Wood Johnson Medical School, New Brunswick, NJ.*

Greater use of pyrethroid insecticides in residential and agricultural pest control has increased the potential for exposure to the general population. The fetus may be at greater risk of developing exposure-related neurological dysfunction due to immature metabolic capabilities and sensitivity to chemical insult during critical developmental windows, although little research has been conducted in the area. Pyrethroids in umbilical cord blood best represent the biologically effective dose received by the fetus during an exposure event in the late third trimester of pregnancy. Previous methods had minimal success in measuring pyrethroid parent compounds and metabolites in serum. Pyrethroid metabolites may be better biomarkers in serum, as parent compounds are rapidly metabolized. A GC-MS/MS method was developed to quantify low concentrations of six pyrethroid insecticide metabolites, cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (cis/trans-DCCA), cis- and trans-chrysanthemum dicarboxylic acid (cis/trans-CDCA), cis-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (cis-DBCA), 4-fluoro-3-phenoxybenzoic acid (4-F-PBA), and

3-phenoxybenzoic acid (3-PBA) in cord serum. The metabolites were separated by DCM liquid-liquid extraction followed by derivatization using hexafluoro-2-propanol and N,N'-Diisopropylcarbodiimide. Cord blood samples were obtained from 74 individual live births that occurred at Robert Wood Johnson University Hospital. Of the six metabolites, 3-PBA, trans-DCCA, cis-DCCA, and 4-F-3-PBA were present in 45 cord serum samples, with 81.4%, 11.6%, 2.3%, and 2.3% above LOD, respectively. Average serum concentrations were 0.16 ng/mL ±0.32, 0.24 ng/mL ±0.19, 0.16 ng/mL, and 0.82 ng/mL, respectively. This method was successful in detecting and quantifying pyrethroid metabolites in cord serum. 3-PBA was the most prevalent metabolite and has potential use as a representative exposure biomarker. This work is supported by the NIEHS through: P30ES005022, T32ES019854 and T32ES007148.

1136 RapidFire-Ion Mobility-Mass Spectrometry Supports High-Throughput Assessment of the Human Exposome in Blood and Urine

E. S. Baker[3], X. Zhang[3], T. O. Metz[3], D. G. Thomas[3], D. Mehinagic[3], J. Ma[3], R. Renslow[3] and J. G. Teeguarden[3]. *[1]Computational Biology, Pacific Northwest National Laboratory, Richland, WA; [2]Health Effects and Exposure Science, Pacific Northwest National Laboratory, Richland, WA and [3]Integrative Omics, Pacific Northwest National Laboratory, Richland, WA.*

The goal of conducting comprehensive assessment of human exposure to endogenous and exogenous chemicals, across time and in large populations poses new challenges to conventional analytical methods. These challenges include extensive expansion of libraries of chromatographic features for targeted analyses, applicability to broad sets of chemistry, large reductions in analysis time for library building and sample analysis. Here we demonstrate the first application of an ultra-fast RapidFire separation system linked to an Ion Mobility-Mass Spectrometer (IMS-MS) platform for characterization of the human exposome. This system, along with a custom informatics pipeline, was used to develop a library of more than 500 compounds including endogenous metabolites and xenometabolites. Using the RapidFire-IMS-MS system each sample could be analyzed with one solid phase extraction cartridge in ten seconds (360 analyses/hr), allowing multiple cartridge analyzes in less than a minute. Detection limits for xenometabolites in serum and urine ranged from the 500 picomolar to 100 nanomolar depending on the molecule studied and standard curves were observed to be linear for tested compounds. Isomeric metabolite difference were also observed in a study analyzing 100 patients, consisting of 50 control and type 1 diabetic patient sibling pairs, with three SPE cartridges in both positive and negative polarity. The isomeric differences were identified with MS/MS after IMS separation allowing full characterization of each compound.

1137 Differential Bisphenol A Levels in Sewage Wastewater Effluents from Communities Located within Metropolitan Detroit Area

J. M. Santos[1,3], A. Joakim[1], D. Putt[1], B. Hosea[1], D. Kaplan[1], M. Jurban[2] and H. Kim[1]. *[1]Detroit R&D, Inc., Detroit, MI; [2]Detroit Water and Sewage Department, Detroit, MI and [3]Physiology, Federal University of Goias, Jatai, Brazil.* Sponsor: H. Kim.

The rapid growth of industrial applications of bisphenol A (BPA) accounted for a global production rate of about 5 million tons in 2010. BPA is abundant in the aquatic environment because it is continuously released from chemical manufacturing processes, human body waste and the disposal of synthetic products into sewage effluent. Therefore, the aims of this study are to measure BPA levels in sewage wastewater at three different collection points over a one year period, and to compare the levels of BPA to 8-isoprostane, a human derived fatty acid, found in sewage wastewater. Pre-treated sewage samples collected from 3 different source points located in different communities in the metropolitan Detroit area (JEF, NIE, OAK) were provided by the Detroit Water and Sewerage Department over a one year period. Human urine samples were also used in the study. BPA and 8-isoprostane were measured using an ELISA kit from Detroit R&D, Inc. BPA levels from the same collection point oscillated more than 10-fold over 12 months (0.8 µg/L to up to 10 µg/L). Moreover, there were seasonal fluctuations in BPA levels, higher in fall and early winter compared to the rest of the year. LC/MS/MS confirmed ELISA results from collection points that presented highest oscillation of BPA among months. The concentration of BPA in sewage wastewater was ~100-fold higher than the concentration of 8-isoprostane, while urinary concentration was ~20-fold, suggesting that the majority of BPA in sewage wastewater might be not be derived from human urine. Our results suggested that BPA levels in sewage water seasonally varied and differentially detected among the three communities in metropolitan Detroit.

1138 Troubleshooting Challenges Arising from Contamination during the Translation of a Published Method (LC-MS/MS) for Measurement of Bisphenol A in Biological Fluids at Trace Level

B. Buscher[8], D. van de Lagemaat[8], W. Gries[4], D. Beyer[2], D. A. Markham[7], R. A. Budinsky[7], S. S. Dimond[6], R. V. Nath[5], S. A. Snyder[3] and S. G. Hentges[1]. *[1]American Chemistry Council, Washington, DC; [2]Bayer Pharma, Wuppertal, Germany; [3]Covestro LLC, Pittsburgh, PA; [4]Currenta GmbH & Co, Leverkusen, Germany; [5]SABIC Research & Technology, Bangalore, India; [6]Saudi Basic Industries Corporation, Bedford, NH; [7]The Dow Chemical Company, Midland, MI and [8]TNO Triskelion BV, Zeist, Netherlands.*

An accurate and sensitive analytical method is critical to proper measurement of Bisphenol A (BPA) in biological fluid samples in support of bio-monitoring studies. It is well-known that following controlled BPA oral dosing humans excrete BPA into urine, predominantly as its conjugated forms. If a substantial amount of unconjugated (free) BPA is observed (e.g., >1% of the dosed amount), it is suspected that this may be due to exogenous sources introduced during sample collection, preparation, and analysis, and/or by the hydrolysis of BPA conjugates to BPA. In order to understand these anomalies, a published LC-MS/MS method for quantification of free and total BPA in urine samples was replicated in another laboratory to carefully evaluate factors that could contribute to erroneous results. Accordingly, urine samples were subjected to enzymatic hydrolysis for de-conjugation prior to BPA analysis. During analysis, it was found that all of the blank solvents, inclusive of water and the UPLC system itself, contributed to 12C-BPA contamination, and that appropriate trouble shooting was needed prior to conduct of actual studies. The final method linearity range was 0.05 to 25 ng/mL. The LOQ was 0.1 ng/mL for free BPA and 0.2 ng/mL for total BPA, respectively. The method was applied to urine samples derived from 20 volunteers. It was concluded that, while in principle, reliable estimation of trace levels of BPA is possible, significant care needs to be taken to avoid contamination. Further, as a rule of thumb, any indications of substantial free BPA (>1%) compared to total BPA calls for an investigation of potential contamination and/or conjugate hydrolysis.

1139 Bioactive Bisphenol A Is Unmeasurable in Serum during Pregnancy at Exposures Typical for the US and Canada

M. I. Churchwell[1], N. C. Twaddle[1], D. R. Doerge[1] and J. G. Teeguarden[2]. *[1]Biochemical Toxicology, US FDA, Jefferson, AR and [2]Health Effects and Exposure Science, Pacific Northwest National Laboratory, Richland, WA.*

Bisphenol A (BPA), a monomer used in the manufacture of polycarbonate plastics and epoxy can liners, is found in trace quantities in some consumer products and food containers. Despite its very low oral bioavailability and rapid elimination, multiple reports of unexpectedly high BPA concentrations in the serum of pregnant mothers or cord blood raised questions about BPA exposures during pregnancy. Hypotheses explaining these findings include altered pharmacokinetics during pregnancy, unique or higher exposure during pregnancy, limitations of these studies, such as contamination, or uncommon exposure settings. We recruited 30 healthy mid-term pregnant women and evaluated total BPA exposure over a 30 hour period comprising one-half day in the field and one day in a clinical setting. Twenty volunteers were selected for high consumption of canned food or cash register receipt handling. BPA and BPA metabolites were measured in serum and total BPA measured in matching urine samples. Twenty of the thirty volunteers had total daily exposures equal to or exceeding the US Mean, and six volunteers exceed the 75th percentile. Women working as cashiers did not have higher BPA exposure or serum concentrations. Exposures were higher in the clinical setting than the field setting. BPA was detected in some serum samples (0.246–0.513 ng/ml), but showed no relationship to total BPA in corresponding urine samples, no relationship to total exposure, and had aglycone fractions of 60–100%, and therefore could be attributed to contamination during collection or handling. We conclude that at exposure levels typical of US and Canadian pregnant women, internal exposures to BPA are below limits of detection.

1140 Urine and Serum Biomonitoring of Exposure to Dietary Estrogens in Pregnant Women in the United States: Soy Isoflavones and the Mycotoxin Zearalenone

S. C. Fleck[1], D. R. Doerge[1] and J. G. Teeguarden[2]. [1]*Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR and* [2]*Health Effects and Exposure Science, Pacific Northwest National Laboratory, Richland, WA.*

Fetal development is a period of potentially greater sensitivity to chemical exposure. Exposure of the fetus to chemicals with the potential to bind and interact with hormone receptors, for example estrogen receptors, is thought to be of particular concern. To characterize the potential estrogenic load of a select group of dietary estrogens during pregnancy, we recruited 30 healthy mid-term pregnant women from the greater Salt Lake City area and evaluated exposure to several dietary estrogens over a 30 hour period compromising one-half day in the field and one day in a clinical setting. Biomonitoring of the dietary estrogens genistein (GEN), daidzein (DDZ) and equol (EQ) as well as the mycoestrogen zearalenone (ZEN) and its congeners was conducted using UPLC-MS/MS. Urinary concentrations of GEN, DDZ and EQ in pregnant women resemble levels reported for general adult US population. Some spot samples exceeded the 95th percentile. Aglycone levels of GEN and/or DDZ in serum could be measured in 6 out of 30 subjects and ranged between 0.6 to 7.1 nM. Urine to serum ratios of 13 (GEN), 47 (DDZ) and 180 (EQ) were calculated for use in estimating serum concentrations from urine biomonitoring data. Despite a rather low dietary intake of corn-based food with the potential for mycotoxin contamination, we are the first to report that zearalenone and its reductive metabolite α-zearalenol are present in pregnant women (11 out of 30 volunteers) with levels near the limit of quantification. Overall, we found evidence of internal exposure in pregnant women to multiple estrogens present naturally in the food supply, which together contribute to the total estrogen level in the body.

1141 Simultaneous Determination of Androgens and Estrogens in Human Urine by Liquid Chromatography

D. C. Escobar-Wilches[2], M. L. Lopez-Gonzalez[2], L. E. Torres-Sanchez[1] and A. Sierra-Santoyo[2]. [1]*Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico and* [2]*Departamento de Toxicologia, Cinvestav-IPN, Mexico City, Mexico.*

Endocrine-disrupting chemicals may alter androgens and estrogens balance. Methods commonly used to measure these hormones have some disadvantages. Liquid chromatography (LC) analysis provide high specificity, sensitivity, and versatility. The objective was to develop an analytical method to simultaneously determine estrogens and androgens and their metabolites by LC in human urine. A ZORBAX Eclipse XDB-C18 column (150 X 4.6 mm ID, 5 µm particle size) was used. Analytes were resolved using a HPLC gradient program of four steps of acetonitrile, methanol and water at 0.6 ml/min and 21 °C. The duration of program was 50 min. Analytes were detected at 225 nm (estrogens) and 247 nm (androgens). Diethylstilbestrol (1.5 nmol) was added as internal standard. Analytes were extracted from an aliquot of 4 ml using C-18 SPE cartridges and then residue was reconstituted and this was subjected to enzyme hydrolysis. Then hydrolyzed analytes were extracted and these were dissolved in methanol and analyzed by LC/DAD. The identity of metabolites was confirmed by retention time and UV spectra of standards. The gradient program of solvents established allowed an adequate resolution and definition of 15 estrogens and 8 testosterone metabolites. The linearity was verified in a range from 5 to 200 ng for all analytes (r>0.9997). LOD ranged from 6.71 to 17.38 ng/ml for all analytes. Absolute recoveries ranged from 80 to 120% with 2-10% coefficient variation. Analytes 6β-hydroxytestosterone (-OHT), 2β-OHT, testosterone, E3, 16α-OHE1, 4-OHE1, 2-OHE2, 3-MeOE1, E1, and E2 were the most current metabolites found in urine of volunteers. These results suggest that this method represents an excellent alternative to simultaneously analyze estrogens and androgens in urine and may be used to evaluate alterations on estrogens and androgens balance in studies on endocrine disruption, steroid metabolism, and hormone-dependent pathologies.

1142 Phthalate Exposure during Puberty: A Window for Interaction Between the Endocrine and the Immune System

N. Lambrechts[6], C. Franken[5], E. Govarts[6], E. Den Hond[6], V. Nelen[1], I. Loots[5], W. Baeyens[4], I. Sioen[2], T. Nawrot[3], L. Bruckers[3], S. Voorspoels[6] and G. Schoeters[6]. [1]*PIH, Antwerp, Belgium;* [2]*University of Ghent, Ghent, Belgium;* [3]*University of Hasselt, Hasselt, Belgium;* [4]*VUB, Brussels, Belgium;* [5]*Biomedical Sciences, University of Antwerp, Antwerp, Belgium and* [6]*Environmental Risk and Health, VITO, Mol, Belgium;* [7]*Social Sciences, University of Antwerp, Antwerp, Belgium*. Sponsor: L. Aylward.

Puberty is generally considered as a critical window of exposure due to hormonal changes that lead to development of the adult phenotype. We investigated in a study of adolescents in Flanders (Belgium) whether phthalate exposure is associated to asthmatic and allergic outcomes and to oxidative stress. The study sample of 418 14-15 year olds was recruited as part of the Flemish Environment and Health Surveillance program (FLEHS). Data from 2 subsequent cohorts were pooled and harmonized (2008-2009 and 2013). In spot urine samples the metabolite concentration of phthalates DEHP (sum of MEHP, MEHHP and MEOHP) and DBP (MnBP) was measured. As a marker of systemic oxidative stress we analysed 8-hydroxy-deoxyguanosine (8-OHdG) in urine. Information on doctor-diagnosed asthma, hay fever and respiratory allergy was collected using a standardized, self-reported questionnaire. Multiple linear or logistic regression models were used to calculate the change of effect to phthalate exposure. All models were adjusted for sex, age, cohort, smoking status and familial allergy. The systemic oxidative stress level was positively associated to the body burden of both phthalates. Interestingly, the estimated 8-OHdG increase due to phthalate exposure was consistenly higher in girls than in boys. Further, younsters with higher metabolite concentrations of the high molecular weight compound DEHP also had a higher risk of doctor-diagnosed asthma, without an apparent effect modification of gender. The low molecular weight DBP was not associated with increased asthmatic or allergic outcomes. These data indicate that exposure to HMW phthalates during puberty is associated with a higher risk of asthma and that a possible underlying mechanism may be the induction of oxidative stress. This is in line with the observation in mechanistic studies of an adjuvant effect of some phthalates on allergic sensitization. The sex dependent increase of 8-OHdG suggests the endocrine disrupting mode of action of phthalates in adverse outcomes of the immune system.

1143 Metals & Organics Biomonitoring in Female Vietnamese Electronic Waste Recyclers, General Population, & US NHANES

A. Schecter[4], H. T. Quynh[1], W. Shropshire[2], J. Lanceta[4], J. Kincaid[4] and L. Birnbaum[3]. [1]*Center Ecological Agriculture, Hanoi, Viet Nam;* [2]*Epidemiology, University of Texas, Houston, TX;* [3]*NCI, NIH, Research Triangle Park, NC and* [4]*Pharmacology & Toxicology, University of Louisville, Louisville, KY;* [5]*School of Medicine, University of Louisville, Louisville, KY.*

No systematic study of the general population in organics and metals similar to US NHANES, representing the general US population, exists in Vietnam. Here, we report positive findings from a biomonitoring study for metals and organics of female electronic waste (e-waste) recyclers in rural northern Vietnam and comparisons not exposed to e-waste, and age matched US NHANES general population for comparison. Whole blood, serum and urine were collected from 40 Vietnamese recyclers and 20 Vietnamese comparisons and analyzed at CDC laboratories of A. Sjodin and K. Caldwell. Organics and metals associated with e-waste were measured. In addition, pesticides, other metals, and PCBs were measured to provide data on compounds associated with e-waste and other health related compounds present in the mixtures in these persons. Most PCBs, pesticides and most metals were not statistically significant between recyclers and Vietnamese comparisons. However, PBDEs and urine mercury were higher in recyclers vs comparisons. Blood and urine lead were significantly higher in e-waste recyclers vs comparisons. Vietnamese median levels were noted to be higher than NHANES for blood lead and mercury, urine arsenic species and cadmium. For organics, PBDE-47 and 100 were lower in Vietnamese vs US, but BDE153, 154, -183 and 209 were higher in Vietnamese than NHANES. PCB 138/158 were lower in Vietnamese. Improved occupational safety measures should reduce the levels of PBDEs, lead, and cadmium in recyclers. Both occupational and environmental prevention measures may reduce exposure to arsenic, cadmium, and lead in the general population. Where environmental contamination exists, as in the case of arsenic, change in the depth of household water wells has been known to decrease arsenic exposure.

1144 Dose Reconstruction in Workers Exposed to Permethrin and Cypermethrin and Determination of Biological Reference Values Using a Toxicokinetic Model

J. Côté and M. Bouchard. *Environmental and Occupational Health, University of Montreal, Montreal, QC, Canada.*

Recently, a toxicokinetic model has been developed to describe the time profiles of biomarkers of exposure to permethrin and cypermethrin pyrethroids. Model parameters were determined on the basis of urinary excretion time courses in volunteers exposed to cypermethrin. In the current work, parameters have been refined using novel blood and urinary metabolite data (trans-/cis-DCCA and 3-PBA) in volunteers exposed to both permethrin and cypermethrin. The model then served to reproduce the time courses of biomarkers of exposure in agricultural workers exposed to these two pyrethroids (n = 46 profiles) and predict corresponding absorbed doses. It also allowed inferring on main route of exposure in workers during the study period. The modeling confirmed that the kinetics of permethrin and cypermethrin was similar on the basis of blood and urinary time course data in exposed volunteers. It also showed that simulations of an increased exposure mostly by the oral route during the 3-day biomonitoring period provided the best fit to the urinary excretion time courses of most workers. This is compatible with an inadvertent oral exposure during work periods. According to best fit scenarios, absorbed doses in workers reconstructed with the model reached a maximum of 2.4 µg/kg bw/d and were thus below the absorbed dose limits associated with an exposure to the reference dose values (RfD) established by the U.S. Environmental Protection Agency (0.1 and 0.25 mg/kg bw/d for cypermethrin and permethrin, respectively) and the Acceptable Operator Exposure Level (AOEL) set by the European Commission (0.06 mg/kg bw/d for cypermethrin). Modeling was further used to derive biological reference values (trans-/cis-DCCA and 3-PBA metabolite levels) not to exceed to prevent health effects based on simulations of an exposure to the cypermethrin and permethrin RfD values. Respective values of 12 and 29 nmol/kg bw/d of trans-DCCA, and 5 and 13 nmol/kg bw/d of 3-PBA were obtained. None of the workers presented values above these threshold limits.

1145 Rapid Determination and Validation of S-phenylmercapturic Acid in Workers' Urine Using a Dilute-and-Shoot Approach and Ultra Performance Liquid Chromatography Coupled with Electrospray Tandem Mass Spectrometry (UPLC-MS/MS)

S. Gagné and J. Poulin. *IRSST, Montréal, QC, Canada.*

Worker benzene exposure assessments are evaluations that must be performed in several contexts as well as regular occupational monitoring of workers. Situations such as spillage, inadequate protective equipment or non-functional apparatus, to name a few, lead to undesired occupational exposure. Biological monitoring is commonly used to determine the exposure level. S-phenylmercapturic in urine is a biomarker of choice for conducting biological monitoring of benzene with high specificity. When undesired situations occur, decision makers want to react as promptly as possible in order to take the best possible decision and maintain a safe occupational health environment. The analytical method is a critical step in the decision-making process. It must be as rapid as possible while being reliable at the same time. Improvement of methods is always a subject of interest to those conducting biological monitoring. Ultra performance liquid chromatography coupled with tandem mass spectrometry has been used to provide a rapid determination of S-phenylmercapturic acid in workers' urine while being fully validated. The analysis, conducted in electrospray negative mode, used a simple dilute-and-shoot approach in order to reduce the time needed to prepare worker urine samples. Targeting only S-phenylmercapturic acid by ultra performance liquid chromatography also resulted in a 10- to 15-fold reduction in run time compared to some published methods. The method was developed with a dynamic range between 20 nmol/L and 1013 nmol/L to cover the entire BEI. The limit of detection and the limit of quantification are respectively 7 nmol/L and 23 nmol/L. The intra-day and inter-day precisions are <4% for all concentration levels tested. The accuracy was within an appropriate range of 99 ± 2% at a target level of 178 nmol/L, completing the full method validation in line with the mindset of the AIHA-LAP, LLC. The validated method has been used successfully in the inter-laboratory G-EQUAS program, where the measured concentrations matched the expected concentrations. The method was also used to determine the concentration of S-phenylmercapturic acid in the urine of workers involved in a chemical spillage. The method is unequalled in terms of efficiency combined with data reliability. It is the method of choice for rapid determination of S-phenylmercapturic acid in workplace contexts.

1146 Population Urinary Toxicokinetic Approach for Human Biomonitoring

D. Barbeau[2], M. Neely[1], A. Balestra[2], M. Marques[2], J.-B. Woillard[3] and A. Maitre[2]. [1]*Children's Hospital, University of Southern California, Los Angeles, CA;* [2]*EPSP-TIMC (CNRS UMR 5525), University of Grenoble, Grenoble, France and* [3]*INSERM UMR 850, University of Limoges, Limoges, France.* Sponsor: C. Viau.

We developed a population toxicokinetic approach to model exposure biomarker concentrations in spot urine samples and study inter-individual differences. This was applied to Fluorene exposure from tobacco consumption. We studied 12 smokers. We recorded the timing of all cigarettes (mean=12/subject) and urinations (mean=10/subject) during 24 hours. In an aliquot from each voiding, we quantified 1-, 2- and 3-hydroxyfluorene by GC-MS. We used the Pmetrics software to build a non-parametric population model from the data. A Fluorene dose of 1669 ng was applied to each cigarette(*). Models were compared by AIC. To avoid an incorrect error model arising from cumulative summing of urinary excretion, we reset amounts in the elimination compartment after each measurement to simulate the emptying of the bladder. The final model included a bioavailability term to reproduce the variability of Fluorene doses coming from different cigarettes. First-order absorption was superior to Michaelis-Menten or Weibull equations. A peripheral tissue distribution compartment improved predictions, as did inclusion of non-zero initial conditions in the central compartment taking into account residual exposure coming from previous cigarettes. R^2 of scatter plots of individually predicted vs. observed concentrations reached 0.809, 0.823 and 0,836 for 1-, 2- and 3-hydroxyfluorene, respectively. Bias = -122, -287 and -252 ng. % RMSE = 45.9, 40.3 and 39.8. Finally, apparent urinary half-lives of metabolites (median [90th perc.]) = 2.0 [3.2], 1.7 [8.1] and 4.0 [13.9] h. The model allowed toxicokinetic simulation of Fluorene from urinary data only, and population approach highlights the inter-individual variability. These results could be extrapolated for others environmental toxicants in order to study individual susceptibility. (*) Moir D. et al. A comparison of mainstream and sidestream marijuana and tobacco cigarette smoke produced under two machine smoking conditions. *Chem. Res. Toxicol.*, 2008, 21(2):494-502

1147 Para-Dichlorobenzene Exposure Is Associated with the Alteration of Thyroid Functions in US Adults and Adolescents

Y. Wei[1] and J. Zhu[2]. [1]*Department of Community Medicine, Mercer University School of Medicine, Macon, GA and* [2]*Department of Mathematics and Computer Science, Fort Valley State University, Fort Valley, GA.*

Our previous studies have found an association of urinary concentrations of 2,5-dichlorophenol (2,5-DCP), a reliable biomarker for para-dichlorobenzene (p-DCB) exposure from indoor environments, with obesity, metabolic syndrome and diabetes. To explore endocrine actions that could be elicited by p-DCB exposure, we examined in this study the potential relationship between urinary 2,5-DCP and thyroid functions in adults and adolescents who participated in the 2007-2012 National Health and Nutrition Examination Surveys. A total of 2477 adults (aged 20-79 years) and 612 adolescents (aged 12-19 years) were analyzed for the association between urinary 2,5-DCP and serum thyroid measures, including thyroid-stimulating hormone (TSH), total thyroxine (T4), free T4, total triiodothyronine (T3), free T3, and thyroglobulin (Tg), using generalized linear regression models with the adjustment for age, sex, race/ethnicity, poverty status, education, physical activity, cigarette smoking, alcohol consumption, BMI, urinary iodine, and urinary creatinine. We observed significant inverse relationships between urinary 2,5-DCP and TSH in adult participants, but positive relationships with TSH in adolescents. Urinary 2,5-DCP was also found being positively associated with free T4 in both adults and adolescents, and with Tg only in adolescents. These results suggest that exposure to p-DCB, measured as urinary 2,5-DCP, could alter thyroid gland functions in humans, which may contribute to the potential metabolic disorders that were observed for this chemical. More detailed epidemiologic and mechanistic studies are needed to further explore these interactions.

1148 Brain-on-a-Chip Multi Chamber Platform to Study Cellular Migration

D. Pamies[3], E. Lavell[3], H. Guerrero-Cazares[3], H. Thomas[3], H. Helena[3], B.-P. Anna[4], A. Quinones-Hinojosa[3], L. Andre[5] and O. Killic[3]. *[1]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD; [2]Department of Neurosurgery, Johns Hopkins University, Baltimore, MD; [3]EHS, Johns Hopkins University, Baltimore, MD; [4]Joint Research Centre, European Commission, Ispra, Italy and [5]Yale Systems Biology Institute, Yale University, West Haven, CT.*

Brain structure and neuronal network formation require neuronal migration in order to incorporate neurons and glia cells at the right location after their early differentiation. Migration is a complex process that involves numerous signals and necessary expression of appropriate receptors in precise time-frames for axon guidance of the cells. Classic cell migration *in vitro* assays consist of seeding the cells on a bare surface (Glass or plastic), or on a surface coated with matrix (collagen type I or type IV, fibronectin, laminin, etc.) not representing the *in vivo* situation. This study presents a brain-on-chip platform that mimics various aspects of the developing and adult brain and allows for neuronal migration studies in a more *in vivo* representative way. The platform includes on-chip differentiation of pluripotent human embryonal-carcinoma cell line (NTera2) progenitors over an extended period (>1 month) to form a brain-like environment containing neuronal clusters and glial cells, separated from an upper layer by a perforated PDMS membrane, which is seeded with brain microvascular endothelial cells to form a monolayer with tight junctions mimicking the blood-brain barrier. The platform is used to study neuronal migration in stable gradients formed over long distances (>15 mm). Fetal neural progenitor cells introduced into the chip are shown to respond to two known factors involved in neuronal development, a chemoattractant (CXCL12) and a chemorepellent (SLIT2), by migrating in a more persistent manner compared to a control case where there is no brain-like environment. This supports observations that signaling from surrounding tissue may play a role in how cells *in vivo* detect and respond to distant chemoattractant sources. This brain-chip provides a practical model system for studying how endogenous factors cross the blood-brain barrier (such a drugs or chemicals) and affect neuronal migration and brain development.

1149 Effects of Conazole Fungicides on Spontaneous Activity in Neural Networks

B. S. Lynch[2], C. M. Mack[1] and T. J. Shafer[1]. *[1]Integrated System Toxicology Division, NHEERL, US EPA, Research Triangle Park, NC and [2]Department of Biology, North Carolina Central University, Durham, NC.*

Hexaconazole (HEX), Tetraconazole (TET), Fluconazole (FLU), and Triadimefon (TRI) are conazole fungicides, used to control powdery mildews on crops, and as veterinary and clinical treatments. TRI, a demethylation inhibitor, is neurotoxic *in vivo*, and previous *in vitro* experiments produced characteristic alterations in neural network mean firing rate (MFR) and bursting patterns measured using microelectrode array (MEA) recordings. Other tested conazoles reduced MFR, but effects on bursting activity were not determined. The present study used 48-well MEA plates to assess effects of FLU, HEX, TET, TRI, and Spiroxamine (SPI, a spiroketal-amine fungicide), on cell viability, MFR, active electrodes (#AE), synchrony (SYNC) and burst parameters (Interburst Interval (IBI), Burst Duration (BD), Burst Frequency (BF), and Number of Bursts (NB)). Primary cortical cell cultures expressed spontaneous electrical activity after 12-15 days *in vitro* (DIV) in the form of individual spikes and bursts and were tested between DIV 14-34. MFR, bursting and SYNC data were collected, and then analyzed using AxIS 2.1, Axion Biosystems (Atlanta, GA). All compounds were without effect on viability measured by lactate dehydrogenase release assay. IC50 values for effects of SPI, TET, HEX and TRI on MFR were 0.44, 0.72, 1.5, and 31µM (n=3), respectively. FLU was without effect on any activity parameter. The remaining fungicides caused similar concentration-dependent decreases in MFR, #AE, burst parameters, and SYNC, with all network activity being completely inhibited at highest concentrations (10-100 µM). In this study, both conazole and amine compounds had neuroactive effects similar to TRI on MFR, and altered bursting characteristics in the neural network. Further investigations are needed to determine the underlying mechanisms by which these chemicals modify cortical network function. (This abstract does not reflect the policies of the United States Environmental Protection Agency)

1150 Further Evaluation of Developmental Neurotoxicity (DNT) Hazard Screening using Neural Networks from Rat Cortical Neurons on Multi-well Microelectrode Arrays

J. Brown, K. Wallace, D. Hall, W. R. Mundy and T. Shafer. *ISTD, US EPA, Research Triangle Park, NC.*

Thousands of chemicals have not been characterized for their DNT potential. Due to the need for DNT hazard identification, efforts to develop screening assays for DNT potential is a high priority. Multi-well microelectrode arrays (MEA) measure the spontaneous activity of electrically excitable cells at multiple sites in a network (a single well) providing sufficient throughput as well as high-content data. The current study examined the ontogeny of spontaneous activity in cortical neural networks from Long Evans rat pups (0–1 day old) after developmental exposure to a set of compounds with *in vivo* evidence of developmental neurotoxicity. Primary cultures are comprised of both excitatory (glutamatergic) and inhibitory (GABAergic) neurons and glial cells. Activity was monitored over 12 days *in vitro* (DIV). Previous evaluation of this assay by screening 36 compounds indicated that MEAs may be useful for screening compounds for potential DNT hazard. The present study evaluated 12 additional compounds: 8 positives (amphetamine, chlordiazepoxide, colchicine, aminonicotinamide, bis(tri-n-butyltin)oxide, acrylamide, diethylstilbestrol, PBDE-47) and 4 negatives (Chloramben, cotinine, isoniazid, acetaminophen) at 0–30 µM. Cortical cells were exposed to compound 2 hours after seeding on a 48 well MEA plate. Network activity was recorded for 15 minutes on DIV 2, 5, 7, 9, and 12. Following the DIV 12 recording, viability was assessed by Cell Titer Blue and LDH assays. On DIV 12, positives Bis(tri-n-butyltin)oxide, acrylamide, diethylstilbestrol, PBDE-47,) decreased the mean firing rate (MFR) at concentrations that were not cytotoxic, while chlordiazepoxide, colchicine, and aminonicotinamide decreased MFR concomitantly with cell death. Negative compounds chloramben, cotinine, and isoniazid also decreased MFR and viability. Acetaminophen and amphetamine showed no clear effect on either parameter. Combined with previous results, 31/39 compounds with *in vivo* evidence of DNT alter MFR and/or viability, 6/9 negative compounds did not affect either measure. These results indicate that this assay may be a useful screen for compounds with DNT potential. (This abstract does not reflect EPA policy)

1151 Characterization of Toxcast Phase II Compounds Disruption of Spontaneous Network Activity in Cortical Networks Grown on Multi-Well Microelectrode Array (mwMEA) Plates

J. D. Strickland[1], M. Martin[3], A. M. Richard[3], K. Houck[3] and T. J. Shafer[3]. *[1]Axion Biosystems, Atlanta, GA; [2]ISTD, US EPA, Research Triangle Park, NC and [3]NCCT, US EPA, Research Triangle Park, NC.*

The development of multi-well microelectrode array (mwMEA) systems has increased *in vitro* screening throughput making them an effective method to screen and prioritize large sets of compounds for potential neurotoxicity. In the present experiments, a multiplexed approach was used to determine compound effects on both neural function and cell health in primary cortical networks grown on mwMEA plates following exposure to ~1100 compounds from EPA's Phase II ToxCast libraries. On DIV 13, baseline activity (40 min) was recorded prior to exposure to each compound at 40 µM. DMSO and the GABAA antagonist bicuculline (BIC) were included as controls on each mwMEA plate. Changes in spontaneous network activity (mean firing rate; MFR) and cell viability (lactate dehydrogenase; LDH and CellTiter Blue; CTB) were assessed within the same well following compound exposure. Activity calls ("hits") were established using the 90th and 20th percentiles of the compound-induced change in MFR (medians of triplicates) across all tested compounds; compounds above (top 10% of compounds increasing MFR), and below (bottom 20% of compounds decreasing MFR) these thresholds, respectively were considered hits. MFR was altered beyond one of these thresholds by 322 compounds. Four compound categories accounted for 66% of the hits, including: insecticides (e.g. abamectin, lindane, prallethrin), pharmaceuticals (e.g. haloperidol, reserpine), fungicides (e.g. hexaconazole, fenamidone), and herbicides (e.g., imazapyr, metholachlor). Most changes in MFR occurred in the absence of cytotoxicity; with only 8 compounds causing decreased cell viability. Analysis for enrichment of chemical structural features using ToxPrint identified 19 chemotypes, accounting for ~33% of the hits in the mwMEA assay. All 322 hits, as well as a subset of ~50 inactive compounds, will be rescreened in a concentration-response experimental design to confirm activity and determine potency. The MEA data on acute neurotoxicity will be combined with the results of other ToxCast assays for chemical prioritization purposes. (This abstract does not reflect EPA policy).

1152 Acute and Chronic Exposure to Insecticides Modulates Electrical Activity and Spike Patterns of Primary Cortical Cultures on Multi-Electrode Arrays

M. M. Dingemans, M. G. Schutte, F. M. Wijnolts, R. G. van Kleef, A. de Groot and R. H. Westerink. *Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands.*

Neuronal cultures grown on multi-electrode arrays (MEAs) are increasingly being used to study neurotoxic potential of chemicals, using different exposure paradigms. In this study, effects of acute and chronic exposure to insecticides [α-cypermethrin (CYP) and endosulfan (ES)] are studied in primary cortical cultures on MEAs. Different parameters were derived from the MEA recordings and evaluated for their use to detect (developmental) neurotoxicity [(D)NT]. Cortices from neonatal Wistar rats were dissociated and cultured on MEAs in a multi-well format. Neuronal activity of the cortical cultures was recorded as extracellular field potentials. Baseline neuronal activity of the cortical cultures was recorded before exposure (at DIV7, 10 or 11), after which they were exposed to different concentrations of CYP, ES or DMSO (≤0.1% v/v). Activity was recorded immediately and 3, 7, 10 and 14 days after the onset of exposure. Inhibition and induction of neuronal activity was observed in a concentration-dependent manner by acute exposure to CYP and ES, respectively, both with LOECs in the submicromolar range. Such inhibition by exposure to CYP and induction by exposure to ES could also be observed in chronically exposed cortical cultures. Analysis of the distribution of interspike intervals revealed an increase in interval length by acute exposure to CYP. These data show that effects of acute and chronic exposure to CYP and ES on spontaneous neuronal activity including spike patterns can be detected *in vitro* at (sub)micromolar exposure concentrations. This information can be included in human risk assessment of insecticides for (D)NT, although potential species-specificity needs to be excluded using human models. Funding: European Union [DENAMIC; FP7-ENV-2011-282957].

1153 Non-Invasive, On-Chip Measurements of Neuronal Cell Health and Function upon Low Level Sarin Exposure

N. O. Fischer, H. A. Enright, S. H. Felix, P. Spackman, E. Mukerjee, S. Hok, C. Valdez, D. A. Soscia, K. Kulp and E. K. Wheeler. *Engineering, Lawrence Livermore National Laboratory, Livermore, CA; Nuclear and Chemical Science Division, Lawrence Livermore National Laboratory, Livermore, CA; Physical and Life Sciences, Lawrence Livermore National Laboratory, Livermore, CA.*

Organophosphates (OP) are widely used in industry and agriculture for the protection of livestock and crops against insect disease and damage. OPs also represent the most toxic class of chemical warfare agents (e.g. sarin). These agents act by irreversibly inhibiting cholinesterases (ChE) in both the central and peripheral nervous systems, resulting in hyperactivity of the cholinergic system due to acetylcholine accumulation. While high dose OP exposures have been well documented, little attention has been given to the potential deleterious effects of low dose exposure to OPs. Low dose exposure can impact both individuals in the military and civilians through environmental contamination. In this study we investigated effects of low level sarin exposure on neuronal health and function by assessing both peripheral and central nervous system neurons using a multi-electrode array platform. *In vitro* cytotoxicity was determined for sarin in primary rat dorsal root ganglion and cortical neurons. Upon acute low level exposure, changes in network activity, including a reduction in firing rate, were observed for cortical neurons. In addition, sarin stability under relevant experimental conditions was assessed with 19F NMR. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABS-677822.

1154 Human iPSC-derived Neurons on Microelectrode Arrays (MEAs) for *In Vitro* Neurotoxicity Testing

M. W. de groot, F. M. Wijnolts and R. H. Westerink. *Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands.*

Neurotoxicity testing still relies on expensive, time consuming and ethically debated animal experiments. These *in vivo* experiments are unsuitable for screening large numbers of chemicals and require interspecies translation. Consequently, there is a clear need for (high throughput) *in vitro* test strategies, preferably using human cells. However, human stem cell-derived neurons used to date are not well characterized, require prolonged differentiation and are potentially subject to batch-to-batch variation. Recently, a number of human induced pluripotent stem cell (iPSC)-derived neurons became commercially available that may circumvent these concerns. The present study therefore aims at investigating the usability of mwMEA recordings for neurotoxicity screening with human iPSC-derived neurons. Using immunofluorescent stainings we demonstrate that these human iPSC-derived neurons form mixed neuronal cultures consisting of different types of (excitatory and inhibitory) neurons and supporting astrocytes. Additionally, we demonstrate using multi-well microelectrode array (mwMEA) recordings that these human iPSC-derived cultures develop spontaneous neuronal activity over time. Finally, as proof of concept for neurotoxicity testing, we show that spontaneous neuronal activity can be modulated by different physiological, toxicological and pharmacological compounds. While further characterization and toxicological validation is required to facilitate acceptance of these physiologically-relevant human iPSC-based models, our data indicate that commercially available human iPSC-derived neurons rapidly differentiate into neuronal networks that represent the cellular heterogeneity of the *in vivo* brain, develop spontaneous activity and are amenable for *in vitro* neurotoxicity testing. Funding: National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs; project number 50308-372160) and Faculty of Veterinary Medicine (Utrecht University).

1155 Human StemCell-Derived Neurons for Neurotoxicity Functional Screens

C. J. Strock[1], J. A. Bradley[1], M. Roach[2], K. Gomes[2] and R. Malavarca[2]. *[1]Cyprotex US, LLC, Watertown, MA and [2]PhoenixSongs Biologicals, Branford, CT.*

Neurotoxicity produces significant compound attrition during drug discovery. There are currently no preclinical human models to screen for neurotoxicity. Ion channel and receptor activity assays can be used to predict some seizure potential, but these only focus on specifically measured targets for prediction and may miss responses that rely on a combination of targets. Therefore, the development of a high-throughput *in vitro* assay to screen compounds for electrophysiological liabilities using human neurons would be of great value. Here we demonstrate the use of a 48-well Axion BioSystems microelectrode array (MEA) along with custom data analysis algorithms to screen for neurotoxic liabilities using spike data from HIP-009 human neural stem cells (NSCs) differentiated into mature functional neurons. The data presented demonstrates that these human neurons have robust electrophysiological activity and establish organization and networking capabilities as observed with the development of bursting and synchrony patterns in the spike trains. Human HIP-009 NSCs differentiated into neurons on 48-well Axion MEAs were matured in culture for ~8 weeks. The human neurons were treated at multiple time points with various GABAA antagonists and the electrical activity was then measured on the Axion Biosystems MEA instrument. Similar patterns of change were observed with these cells when treated with GABAA antagonists as have been observed with rat cortical neurons. This includes an increase in firing rate, burst organization and synchrony. Although these cells have promising organization, further work is being done to characterize and expedite their maturation process.

1156 Potential Use of Microelectrode Arrays Using Human iPS-Derived Neuronal Cells to Evaluate Neurotoxicity

A. Okamura[1,2], T. Shirakawa[1,2], K. Nagata[2] and A. Okada[2]. *[1]Consortium for Safety Assessment using Human iPS Cells (CSAHi), Tokyo, Japan and [2]Drug Safety Research Labs., Astellas Pharma Inc., Osaka, Japan.*

Neurotoxicity is one of the most severe, frequent toxicities that should be avoided in the drug development. However, a nonclinical prediction of clinical neurotoxicity has been challenging, especially with an *in vitro* system. In a pharmacological approach, microelectrode arrays (MEA) have been a valuable tool to assess a neuronal activity of a drug candidate with *in vitro* systems. However, a relationship between neurotoxicity and neuronal activity detected with MEA has not been characterized yet. In this study, human induced pluripotent stem (iPS)-derived neuronal and astrocyte cells (Cellular Dynamics International, USA) were co-cultured in probes for MEA. There have been many researches of toxicological evaluations using human iPS-derived cells in the fields of cardiotoxicity and hepatotoxicity, but less in the field of neurotoxicity. After a long term culture, spontaneous firing from cells in each probe was measured with the MEA system (ALPHA MED SCIENTIFIC, Japan). Three neurotoxic compounds (4-aminopyridine, gabazine, NMDA) were then added onto cells, and numbers of spikes and bursts were recorded as neuronal activity. The changes in numbers of spikes and bursts with each compound were analyzed by Mobius software (ALPHA MED SCIENTIFIC,

Japan) and used primarily to evaluate neurotoxicity. Furthermore, evaluations for a pattern analysis of spikes and bursts were conducted as additional endpoints to predict neurotoxicity. This study demonstrates a potential use of the MEA assays with human iPS-derived neuronal cells in predicting clinical neurotoxicity in the drug development.

1157 Multi-Electrode Array (MEA) Recordings for Neurotoxicity Screening of Illicit Drugs

L. Hondebrink[3], A. H. Verboven[2], S. Drega[2], S. Schmeink[2], M. W. de Groot[2], R. G. van Kleef[2], F. M. Wijnolts[2], A. de Groot[2], J. Meulenbelt[3] and R. H. Westerink[2]. *[1]Department of Intensive Care Medicine, University Medical Center Utrecht, Utrecht, Netherlands; [2]Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands and [3]National Poisons Information Center (NVIC), University Medical Center Utrecht, Utrecht, Netherlands.*

Annual prevalence of illicit drug and new psychoactive substance (NPS) use is high, although knowledge on the health risks of many of these substances is scarce. Given the large number of available drugs of abuse, efficient techniques for neurotoxicity screening are required. Recently, cortical cultures grown on multi-well multi-electrodes array (mwMEA) have successfully been used to investigate the neurotoxic potential of chemicals with high-throughput. The current research therefore aims at investigating the usability of mwMEA recordings for neurotoxicity screening of illicit drugs. We first demonstrated the abundance of GABA-ergic and glutamatergic neurons and astrocytes in cortical cultures using immunocytochemistry. Next, using mwMEA we demonstrated that the endogenous neurotransmitters GABA, serotonin, dopamine and nicotine concentration-dependently inhibited neuronal activity, whereas glutamate and acetylcholine increased activity at specific concentrations. Finally, methylenedioxy-methamphetamine (MDMA), 1-(3-chlorophenyl)piperazine (mCPP), amphetamine and the NPS 4-fluoroamphetamine (4-FA) also decrease neuronal activity, with IC50s ranging from 32 µM for mCPP to 190 µM for 4-FA. The present data demonstrate the usability of cortical cultures grown on mwMEA for neurotoxicity screening of drugs of abuse. Compared to conventional, time-consuming and mode of action-specific measurements, mwMEA can quickly provide information on integrated aspects of neurotoxicity and health risks, and potentially fasten the regulation process of these drugs. Funding: NVIC (UMC Utrecht) and the Faculty of Veterinary Medicine (Utrecht University).

1158 Peripheral Nerve-on-a-Chip for Predictive Preclinical Pharmaceutical Testing

J. L. Curley[1,2] and M. J. Moore[1,2]. *[1]AxoSim Technologies, New Orleans, LA and [2]Biomedical Engineering, Tulane University, New Orleans, LA.* Sponsor: J. L. Curley.

Use of 3D cultures and microscale engineered tissues as benchtop models for drug and toxicity screening during preclinical development has rapidly been gaining ground, with the hope of curbing attrition rates and rising costs. The ability to quantify organ-specific outcomes *in vitro* has been exploited successfully for epithelial and metabolic tissues, where metabolites and other soluble analytes constitute the measurable read-outs. For peripheral neural tissue, where bioelectrical conduction over long distances may be the most physiological read-out, application of benchtop models has lagged. Our objective was to develop an organotypic, micro-physiological model mimicking morphology of peripheral nerves and supporting physiological measurements analogous to clinical test criteria. Wide-field fluorescence, confocal, and TEM imaging revealed microengineered neural fiber tracts growing within the 3D volume of the Puramatrix hydrogel. The 3D constructs displayed high degrees of parallel fiber growth, high density, fasciculation, and compact myelination. Most of the neurites are relatively small, on the order of ~1 µm in diameter, and both endogenous and exogenous Schwann cells are seen to migrate along with, encapsulate, and myelinate the outgrowing neurites in 3D. Field potential recordings show population responses with consistent delay, amplitude, and envelope under baseline conditions with stimulation. Application of paclitaxel resulted in decreased compound action potential (cAP) amplitude and increased latency. This suggests functional pathophysiological signatures similar to those seen clinically, with analysis and correlation to structural changes still ongoing. Single-cell intracellular recordings also showed electrically-evoked responses whose rise times were independent of baseline voltage. These data strongly suggest that the observed population spikes are wholly compound action potentials propagating down the microengineered neural fiber tract, with the demonstrated ability to induce and examine external toxic pharmaceutical perturbations previously unseen with *in vitro* models. Our data suggest that we can microengineer neural fiber tracts that resemble the morphology, physiology, and pharmacological responses of peripheral sensory nerve tissue. Results demonstrate the feasibility of developing benchtop physiological models with clinically analogous outcomes for drug screening.

1159 Combining MR Imaging with Microelectrode Array Studies to Further Neural Network Ontogeny Analysis

F. T. Goodfellow, G. Simchick, Q. Zhao and S. Stice. *Interdisciplinary Toxicology Program, The University of Georgia, Athens, GA; UGA Biological Imaging Center, The University of Georgia, Athens, GA.*

Independently, electrophysiology analysis via microelectrode arrays (MEA) and magnetic resonance imaging (MRI) analysis are powerful techniques for studying neural networks *in vitro* and *in vivo*, respectively. Our goal was to use these methods in tandem to provide anatomical connectivity form and function within individual organisms to address adverse outcome pathway analysis. Microelectrode arrays (MEA) are electrophysiological monitoring systems used to investigate neural network ontogeny and have proven useful in toxicological assays. These studies often utilize primary CNS cultures from individual(s) independently and investigate *in vitro* exposure to compounds. Functional connectivity within the CNS can be evaluated with diffusion and functional MRI techniques in animals but rarely linked to *in vitro* functional studies. To understand how *in vitro* neural networks relate to *in vivo* functional connectivity, we examined neural networks of similar origin *in vitro* and *in vivo* circumstances. To this end, we used the chicken embryo model. Aside from being inexpensive and not requiring extensive animal facilities, the chicken embryo is a well-characterized model organism within neural developmental biology and toxicology. We examined *in vitro* neural network ontogeny of chicken embryo CNS tissues with the MEA. Concomitantly, we utilized MRI functional imaging of the chick embryo to investigate neural network ontogeny of the CNS *in vivo*. We found that the neural development could be monitored longitudinally on days 10, 15, and 20 of embryo incubation and that tissues could be harvested from different and potentially affected regions of the developing brain. These developing tissues could be monitored for changes in neural network activity (synchronous bursting). We expect that this side-by-side comparison of neural networks within the same developing embryo will provide the basis for future key events toxicological studies as well as greater *in vitro* to *in vivo* predictive power for other models toxicity within the CNS.

1160 The MED64-Qquad II System Increases Throughput for *In Vitro* MEA Pharmacology Studies with Acute Brain Slices

S. Yasuoka, R. Yamazaki and G. Cheng. *Alpha MED Scientific Inc., Ibaraki, Osaka, Japan.* Sponsor: P. McNutt.

Microelectrode arrays (MEAs) have been widely utilized to measure neuronal activities *in vitro*. The MEA technology offers many unique advantages to investigate neuronal circuitry, interaction and models of learning and memory, development, aging, disease, and neurotoxicity. While several high throughput platforms have been utilized for drug screening with cultured cell applications in recent years, there have been limited platforms designed for acute and culture slice applications. Here we present the capabilities of the MED64-Quad II system, a novel medium throughput MEA designed specifically for acute or cultured slice applications. Acute hippocampal slices from 6-8 week old male ICR strain mice were used and the extracellular signals were recorded at 16 electrodes per each slice simultaneously. LTP assay was performed with 4 slices simultaneously using the MED64-Quad II System. On any given experiment, I/O curves were first obtained from fEPSPs in CA1 in response to electrical current stimulation delivered to the Schaffer collaterals. The acute hippocampal slices were bath- perfused with ACSF heated to 32°C temperature. The high capacitance electrodes (55,000 pF) reliably produced greater than 1mV amplitude fEPSP at less than 30µA stimulus amplitude. The relatively large amplitude fEPSP in response to the relatively low stimulating current is due to the low impedance of the platinum black and carbon nanotube electrodes (10 kΩ at 1 kHz). Baseline amplitude and slope of the fEPSP was recorded for 15 minutes in response to stimulating current set to 30% of the current required to saturate the fEPSP amplitude. Following theta burst stimulation, amplitude and slope were monitored for an additional 60 minutes. LTP and LTD assays were performed with administration of several different drugs including MK-801, NMDA channel blocker and NMDA quantitatively. Pretreatment of MK-801 inhibited LTP. Administration of NMDA resulted in toxic effects such as short term depression of fEPSPs, LTD, or disappearance of fEPSPs (with high doses). The results of this study indicated that the

MED64-Quad II system increases throughput while maintaining high sensitivity to detect tiny electrophysiological signals. It is useful tool for drug discovery, target validation, compound screening, as well as increasing productivity of mechanistic studies, toxicological and pharmacological studies in acute brain slice applications *in vitro*.

1161 AOP-Based Identification of Species-Specific Thyroid Hormone Disruptors Using Human and Rodent Neural Progenitor Cells

K. Dach, S. Giersiefer, F. Bendt, U. Huebenthal, H. Heuer and E. Fritsche. *IUF - Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany.*

It is well-known that thyroid hormones (TH) play a crucial role in brain development. The cell biological effects of TH, especially on human developing brain cells, however, remain elusive. For the mode-of-action-based identification of human TH disruptors with *in vitro* systems based on key events (KE) the precise molecular mechanisms of TH action are a prerequisite. Therefore the aims of this project are to 1) characterize species-specific TH signaling in neural progenitor cells (NPCs), 2) elucidate species-specific TH, T3 (triiodothyronine) and T4 (thyroxine), effects on basic processes of brain development (proliferation, migration, differentiation) and - based on these data - 3) establish a test system to identify TH disruptors. TH signaling components are differently expressed in human and rodent NPCs. Moreover, TH guides NPC proliferation and differentiation in a species-specific manner. Specifically, oligodendrocyte formation is only induced by TH in rodent NPCs, whereas their maturation is accelerated by T3 in human and rodent NPCs. Thus, oligodendrocyte maturation was chosen as an endpoint for studying human NPC TH disruption. In this assay brominated diphenyl ether (BDE)-99 was identified as a TH disruptor in murine but not in human NPCs, while NH-3, a TH receptor antagonist, is a TH disruptor in human NPCs. This work identifies and studies KE of TH disruption during neurodevelopment for the building of AOPs in a species-specific context.

1162 Understanding the Relationship between Alterations in Thyroid Hormone Levels and Subsequent Neurodevelopmental Impacts

L. Brown, T. Pepping and K. He. *Abt Associates, Bethesda, MD.*
Sponsor: D. Hattis.

In this work we aimed to understand the relationship between alterations in thyroid hormone levels and subsequent impacts on neurodevelopment. We evaluated the epidemiological literature attempting to explore how these alterations would impact potentially sensitive life stages such as fetuses, neonates, breast-fed and bottle-fed infants and the offspring of pregnant women. The majority of the literature located evaluated the relationship between thyroid hormone levels in a pregnant mother and subsequent neurodevelopmental impacts in the offspring. The findings support the concept that the first trimester of pregnancy is likely the most sensitive in regards to adverse neurodevelopmental effects resulting from altered thyroid hormone levels and that free thyroxine is likely the best indicator when evaluating potential adverse neurodevelopmental impacts. Additionally, the results indicate that even subtle alterations of thyroid hormone levels within what is often considered a normal range can have adverse neurodevelopmental impacts. The implications of these findings could pertain to a wide variety of endocrine disrupting chemicals which are known to impact thyroid hormone levels.

1163 Comparative Developmental Neurotoxicity of Tobacco Smoke and Nicotine, *In Vivo* and *In Vitro*

F. J. Seidler[2], E. D. Levin[2] and T. A. Slotkin[2]. [1]*Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC and* [2]*Psychiatry & Behavioral Sciences, Duke University Medical Center, Durham, NC.*

Although numerous studies have shown the adverse impact of maternal smoking on brain development, little work has been done at low, second-hand smoke (SHS) exposure levels. We gave tobacco smoke extract (TSE) to pregnant rats throughout gestation, simulating plasma nicotine levels seen with SHS exposure, and compared the effects to comparable doses of nicotine alone, and to ten-fold higher nicotine doses that mimic active smoking. TSE impaired cholinergic and serotonergic synaptic development and function, with effects lasting into adulthood; these effects were greater than those of comparable, low-level nicotine, but more closely resembled those of the higher nicotine dose. Regression analysis showed that the higher nicotine level could account for 45% of the TSE effect in males, but only 13% in females. *In vitro* studies using PC12 cells undergoing neurodifferentiation similarly found that TSE effects exceeded those of comparable nicotine doses but resembled those of much higher nicotine. We then attempted amelioration of TSE effects by coadministration of antioxidants or methyl donors; these did not protect against TSE and the ameliorants actually had adverse effects on neurodifferentiation by themselves. Our studies indicate that tobacco smoke components other than nicotine contribute to developmental neurotoxicity, but do so in part by exacerbating the effect of nicotine; there are strong sex differences in the relative contributions of nicotine vs. other TSE components. Further, presumed neuroprotective amelioration strategies appear to be ineffective against TSE. Support: NIH ES022831, US EPA 83543701.

1164 Neurobehavioral Effects of Exposure to Tobacco Smoke Extract in Rats during Critical Windows of Gestational Development

B. J. Hall, M. Cauley, H. J. White, S. Junaid, A. Kiany, Y. Abreu-Villaça and E. D. Levin. *Duke University, Durham, NC.*

Tobacco smoke exposure during development has been associated with cognitive and neurobehavioral impairments in children. Even low dose exposure from second hand smoke (environmental tobacco smoke) has been associated with developmental toxicity. In previous rat studies, we have shown that developmental exposure throughout gestation to chemicals from cigarette smoke in the form of tobacco smoke extract (TSE) at a dose modeling second hand smoke (TSE delivering 0.2 mg/kg/day) causes adolescent locomotor hyperactivity and cognitive impairment under low-motivating conditions. A follow-up study was designed to examine the relative importance of three windows of exposure: premating, early gestation and late gestation. Female rats were exposed to TSE at a dose delivering 0.2 mg/kg/day (sc) during ten days before mating, during the first ten days of gestation or during the second ten days of gestation via iPrecio pumps. Controls received sc infusions of the DMSO vehicle. The offspring were tested on a battery of behavioral tests starting during adolescence and continuing into adulthood. Tests for anxiety (elevated plus maze) and fear (novelty suppressed feeding) were run in sequence for male and female offspring in all treatment groups. The results thus far with the tests of emotional function show significant effects with TSE exposure during portions of development. TSE exposure during the second half of gestation significantly decreased open arm time on the elevated plus maze. This exposure also significantly decreased the duration of feeding in the novelty suppressed feeding test. Both of these results implying increased anxiety in the late gestation TSE exposed rats. Further testing will shed additional light on the cognitive effects of developmental windows of exposure to TSE. The current results suggest persistent dysfunctions of emotional response after late gestational exposure of rats to TSE.

1165 E-Cigarette Exposure Induces Neuro-Alterations in Mice Exposed During Early Life

D. E. Lauterstein, P. B. Tijernia, K. Corbett, T. Gordon, C. B. Klein and J. T. Zelikoff. *Environmental Medicine, New York University, Tuxedo, NY.*

E-cigarettes, battery-powered devices containing nicotine, glycerin, propylene glycol, flavorings, and other substances are increasing in popularity. The lack of regulations and safety data for these products is a concern; particularly since they are often seen as "reduced harm" products compared to cigarettes, and appeal to vulnerable populations, such as pregnant women. While the developmental neurotoxicity of nicotine is well known, the same is not true for other e-cigarette aerosol components. To evaluate the neurotoxic effects of e-cigarette vapor on pre- and post-natal CNS development and later life outcomes, pregnant 9-10-wk-old C57BL/6 mice were exposed daily (3 hr/d; 5 d/wk, via whole body exposure) throughout gestation and lactation to e-cigarette vapor produced from blu e-cigarettes with (16 mg/ml) and without nicotine. RNA-sequencing was used to complete transcriptome analysis on frontal cortex samples from 1-mo-old female and male offspring. Cortex from female offspring exposed early in life to e-cigarette vapor containing nicotine demonstrated that 1,393 genes were significantly changed ($p<0.01$) when compared to controls, while only 152 genes were significantly changed ($p<0.01$) in age- and treatment-matched male offspring. In contrast, frontal samples from juvenile females exposed to e-cigarette vapor with no nicotine demonstrated a change ($p<0.01$) in 2,630 genes when compared to controls, while 2,615 genes were altered ($p<0.01$) in age- and treatment-matched males. RNA-seq data were imputed into Ingenuity Pathway Analysis (IPA) to analyze and compare between sex and treatment groups. Predicted downstream disease and biological function effects associated with observed gene expression changes were identified and included alterations in cognition, learning, and

memory. As more extensive gene expression changes and IPA-predicted adverse outcomes were seen in female and male offspring in the no nicotine e-cigarette groups than the nicotine containing e-cigarette groups (compared to controls), it does not appear that nicotine is the driving factor for these biological effects. Results from this study demonstrate that e-cigarette exposure during early life alters CNS development potentially leading to chronic neuropathology. NYU NIEHS P30ES000260-50 pilot grant.

1166 Neonatal Exposure to Air Pollution and the Autism Phenotype in the Prairie Vole

J. L. Allen[2], M. E. Sobolewski[2], K. A. Conrad[2], K. B. Morris-Schaffer[2], C. Klocke[2], E. S. Barrett[2] and D. A. Cory-Slechta[2]. [1]*Environmental Medicine, University of Rochester School of Medicine, Rochester, NY and* [2]*Obstetrics & Gynecology, University of Rochester School of Medicine, Rochester, NY.*

Several epidemiological studies link air pollution to increased risk for autism spectrum disorders (ASD), findings that may reflect the inflammatory properties of air pollutants. Our prior studies in neonatal mice exposed to concentrated (10-20 fold) ambient ultrafine particles (CAPS) using the HUCAPS system revealed male predominant white matter abnormalities and enlarged brain ventricles, characteristics of neurodevelopmental disorders, including ASD. This study sought to determine whether CAPS exposure elicited an ASD-like behavioral phenotype in the prairie vole (Microtus ochrogaster), a species, unlike mice, with extensive social/communicative behaviors reminiscent of those that are diagnostic of ASD. To determine susceptibility to neonatal inflammation, voles were injected with lipolysachharaide on postnatal day (PND) 8. Confirmation of a neuroinflammatory insult was observed as a prototypical microglial activation response accompanied by altered social behavior during social conditioned place preference (SCPP) testing. Subsequently, neonatal voles were exposed to CAPS via inhalation from PND4-7 & 10-13 for 4 hrs/day to determine whether CAPS elicits an ASD-like behavioral phenotype. CAPS-exposed male voles show significantly increased localized horizontal movements in an open-field arena. CAPS altered social preference in the SCPP in a sex-dependent manner: CAPS males showed increased social preference compared to air-exposed males, whereas CAPS females showed a significantly increased social choice discordance amongst pair-housed conspecifics during SCPP. Sex-specific differences in social preference may indicate that CAPS targets sex steroid signaling and corresponding physiological substrates important to development of normal social behavior in juvenile prairie voles. Alterations in social behavior and increased preservative motor behavior are reminiscent of diagnostic ASD features, increasing the physiological plausibility of the epidemiological link between early life air pollution exposure and adverse neurodevelopment outcomes. Supported by K12-ES019852 & P30-ES001247.

1167 Gestational PM2.5 Exposure Disrupts Brain Development

C. R. Klocke[2], J. L. Allen[2], M. Sobolewski[2], J. Blum[1], D. Lauterstein[1], J. T. Zelikoff[1] and D. Cory-Slechta[2]. [1]*Environmental Medicine, New York University, Tuxedo, NY and* [2]*Environmental Medicine, University of Rochester Medical School, Rochester, NY.*

Both animal and human studies report developmental neurotoxicity in response to various components of air pollution, including ambient particles ≤2.5µm (PM2.5), a fraction that contains ultrafine particles (≤100 nm; UFP), considered the most reactive component of air pollution. Our previous studies in mice exposed postnatally (human 3rd trimester equivalent) to concentrated (10-20x) ambient UFPs using the Univ. of Rochester HUCAPS system, revealed white matter alterations and ventriculomegaly (enlarged lateral ventricles), poor prognostic indicators for neurodevelopment, and characteristics of multiple disorders including autism and schizophrenia, that, as in humans, are also seen primarily in males. The current study sought to determine if prenatal PM2.5 concentrated ambient particles (CAPs) exposure could similarly adversely impact brain development and produce a similar neuropathological phenotype. Pregnant mice were exposed 6 hours daily to CAPs from Sterling Forest NY via the NYU VACES system or filtered air via whole-body inhalation from gestational days 0.5-16.5. Offspring brains were collected between postnatal days (PND) 11-15 and examined histologically for changes in microglial staining density, number, and activation state using the microglial marker IBA-1 in hippocampus, a region of ongoing neuronal proliferation sensitive to microglial activation state, and in corpus callosum (CC) the largest white matter tract in brain that is critical to interhemispheric integration. Gestational CAPs shifted hippocampal microglia to an activated phenotype in females only, even though total microglial cell counts were not affected in either sex. Additionally, both sexes showed ventriculomegaly and early and excessive myelination in the genu of CC, a region of prefrontal cortex fiber crossing. These findings suggest vulnerability of brain during gestation to UFP/ PM2.5 exposures, and a corresponding potential for increased risk of neurodevelopmental disorders such as autism and schizophrenia. Further, the parallel findings from two different CAPs-generating systems (HUCAPS and VACES) suggests the importance of common exposure elements as opposed to regionally-specific constituents of air pollution as the basis of such effects. T32-ES07026, P30 ES001247 P30 ES000260, March of Dimes.

1168 Neonatal Ultrafine Particulate Matter Inhalation Exposure Adversely Impacts White Matter Development

K. B. Morris-Schaffer, J. L. Allen, C. Wong, G. Oberdorster and D. A. Cory-Slechta. *Environmental Medicine, University of Rochester Medical Center, Rochester, NY.*

Air pollution is a leading environmental health concern that contributes to more than 7 million premature deaths worldwide. Epidemiological evidence suggests that air pollution exposure can influence the development of autism spectrum disorders (ASD), schizophrenia, and cognitive decline. Our prior studies indicate that male, but not female mice, exposed neonatally to concentrated (10-20x) ambient ultrafine particles (CAPS; <100nm diameter) show significantly enlarged lateral ventricles (i.e., ventriculomegaly [VM]), a characteristic of low birth weight, and for later diagnosis of both ASD and schizophrenia. Ventriculomegaly and white matter damage frequently co-occur and significant white matter growth occurs during the neonatal period. We hypothesized that ventriculomegaly seen in neonatal CAPS exposure is a result of aberrant white matter development, a pathology often seen in prematurity, and observed in the brains of individuals with ASD and schizophrenia. To test this hypothesis, C57BL/6 mice were exposed to CAPS during the first two weeks of postnatal life with and without adult re-exposure at levels relevant to those measured in the environment. Mice were euthanized at postnatal days 14 or 270, and brains processed for histological examination. White matter development was assessed by quantitative determination of myelin basic protein (MBP) expression in the corpus callosum and striatum. Consistent with our hypothesis, CAPS-exposed males, but not females, exhibited a significantly smaller corpus callosum, and had reduced MBP expression when measured at PND14. When measured at PND270, the effect of postnatal CAPS in males was attenuated but there was an increase in MBP expression in adult-only exposed CAPS males. In females, neonatal CAPS exposure significantly increased MBP expression at PND14, whereas at PND270, adult-only CAPS exposure resulted in a decrease in MBP expression in striatum. Taken together, our data indicate that neonatal CAPS exposure may adversely impact developing white matter in the corpus callosum and the striatum in a sex-dependent manner. These findings may provide the physiological plausibility for the accumulating epidemiological studies reporting associations between development air pollution exposures and adverse neurodevelopmental outcomes in humans.

1169 Developmental Exposure to Diesel Exhaust Induces an Autism-Related Behavioral Phenotype in Mice

Y. R. Chang[2], E. Kalata[2], T. B. Cole[2] and L. G. Costa[2,3]. [1]*Center on Human Development and Disability, University of Washington, Seattle, WA;* [2]*Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and* [3]*Department of Neuroscience, University of Parma, Parma, Italy.*

Escalating prevalence of Autism Spectrum Disorder (ASD) in recent years has triggered more research interest in investigating environmental risk factors such as air pollution. Epidemiological studies have provided evidence linking prenatal and early life exposure to diesel exhaust, a major component of traffic-related air pollution, to an increased risk of children developing ASD. Altered behaviors have also been reported in mice exposed to high levels (1000 µg/m3) of diesel exhaust (DE) developmentally. The goal of the present study was to assess whether exposure of mice to a more environmentally relevant level (250 µg/m3) of DE would alter behaviors within the three diagnostic domains of ASD, i.e. social interaction, verbal/ nonverbal communication, and repetitive/persistence behaviors. Time mated C57BL/6J mice were exposed to either filtered air (FA) or DE for 6 hours per day, 5 days per week from gestational day (GD) 0 to postnatal day (PND) 21. Pre-weaning behavior assessment included maternal behavior recording, righting reflex, and pup ultrasonic vocalization. Post-weaning behavior assessment consisted of open field, marble burying, T maze, olfactory habituation, social preference test, reciprocal interaction, rotarod, and catwalk. We observed increased repetitive behaviors in both males and females exposed to DE assessed by the T maze and the marble burying tests. In addition, we also found

that DE disrupted verbal and olfactory communication. Other behaviors were not affected by developmental DE exposure. These findings suggests that developmental DE exposure can elicit behaviors consistent with what has been observed in patients with ASD; underlying mechanistic pathways and possible gene-environment interaction should be investigated in future studies (Supported in part by T32 ES007032-37, R25ES021646, R01ES022949 and NICHD U54 HD083091).

1170 Effect of Repeated Juvenile Exposure to Δ9-Tetrahydrocannabinol on the Performance of Adolescent Rats in the Elevated Plus Maze

A. N. Mohammed, N. Alugubelly, B. L. Kaplan and R. L. Carr. *Center for Environmental Health Sciences, Department of Basic Sciences, Mississippi State University, Mississippi State, MS.*

Δ9-tetrahydrocannabinol (Δ9-THC), the main psychoactive constituent of *Cannabis sativa*, is the most widely consumed illicit drug in the world. It is well established that exposure to Δ9-THC during both the prenatal period and exposure during adolescence can lead to persistent behavioral changes. However, exposure of young children is becoming an increasing occurrence, but little work has been done to investigate the persistent effects of childhood exposure. The goal of the present study was to investigate the effects of developmental exposure to Δ9-THC on anxiety-related behaviors in adolescence. Ten day old rat pups were exposed orally to either corn oil or 10 mg/kg Δ9-THC daily for 7 days. On day 29, male and female rat pups were tested in an elevated plus maze. The rats were naive to the testing room and the apparatus and the testing was conducted under high levels of light (~700 lux). While male rats exposed to Δ9-THC spent more time in the open arms, both male and female rats exposed to Δ9-THC had similar percent open arm entries and percent time spent in the open arms as compared to their respective controls. These data suggest that juvenile exposure to Δ9-THC does not result in any significant changes in anxiolytic-type behavior in adolescent rats.

1171 Migration of Human Neural Crest Cells as Functional Endpoint to Screen for Developmental Neurotoxicity

J. Nyffeler, X. Dolde and M. Leist. *Doerenkamp-Zbinden Chair for In Vitro Toxicology and Biomedicine, University of Konstanz, Konstanz, Germany.*

Introduction: Neural crest cells (NCCs) are a transient stem cell population arising at the time of neurulation. NCCs migrate to various body parts and differentiate into cells of the peripheral nervous system, melanocytes as well as craniofacial bones and other structures. Therefore, failure of NCC to migrate can lead to severe developmental defects, so called neurocristopathies. Aim: We developed an *in vitro* test system using human NCCs and screened an 80-compound library of potential environmental toxicants for compounds interfering with NCC migration. Methods: NCCs were differentiated from human pluripotent stem cells and cryopreserved until further use. For the cMINC migration assay, cells were seeded in plates containing silicon stoppers to create a cell-free circular area. Removal of the stoppers allowed the start of migration. After 48 h, the number of viable cells in the circular zone was quantified by high-content image analysis. The whole library, originally assembled and published by the national toxicology program of the US (NTP), was tested in three independent biological experiments at the highest non-cytotoxic concentration (≤ 20 μM). Compounds causing > 20% migration inhibition were re-ordered for confirmation and concentration-response curves were obtained. Results: Internal quality controls lead to consistent results and all negative controls were correctly classified. The screen identified 26 compounds as potentially migration-inhibiting, of which 22 were confirmed upon retesting. Among these migration-inhibiting compounds, seven were organophosphates, five were drug-like compounds, three were organochlorines and three were polybrominated flame retardants. Moreover, there were three other pesticides, and an industrial chemical. Conclusions: The cMINC assay yielded stable and reproducible results. Comparison to data already available on the library compounds from *in vivo* testing and other screens suggested a high sensitivity of our assay. We expect that a battery of related developmental toxicity tests may be used to generate alerts on potential toxicants, and to prioritize them for further testing.

1172 Toxic Effects of Astemizole on Neurite Growth and Synaptogenesis of CNS Neurons

T. Shirao[1], N. Koganezawa[1], Y. Ishizuka[1], H. Yamazaki[1], K. Hanamura[1] and Y. Sekino[2]. [1]*Department of Neurobiology and Behavior, Gunma University Graduate School of Medicine, Maebashi, Japan and* [2]*Division of Pharmacology, National Institute for Health Sciences, Tokyo, Japan.* Sponsor: Y. Fueta.

The study of the adverse effects of various drugs on human central nervous system is difficult. In addition it is difficult to extrapolate the neuronal toxicity of drugs from behavior analysis using animals which have far simpler neuronal network than humans. Recent advances in human-induced pluripotent stem cells (hiPSCs) offer new possibilities for biomedical research and clinical applications. Therefore we sought to use human iPSC derived neurons for the assessment of neuronal toxicity of various drugs. In this study, we first compared the developmental processes of human iPSCs-derived neurons (hiPS-neurons) and rat primary cultured neurons focusing on their neurite growth at the early development stages. The polarization of neurite into axon occurs more slowly in hiPS-neurons than rat neurons, and the axonal growth speed of hiPS-neurons remains similar to dendrites or neurites even after the polarization. We then examined the effects of E-4031, chromanol 293B, astemizole, cisapride, quinidine, dl-sotalol, terfenadine and moxifloxacin. Astemizole significantly inhibited the neurite growth of both human and rat neurons. We further analyzed effect of astemizole on synaptogenesis of rat neurons using drebrin cluster density as a marker of mature synapses. Astemizole decreased the synaptogenesis of rat neurons. These result indicated that the neuronal culture system is useful for extrapolating the neuronal toxicity of drugs on human central nervous system.

1173 Comparing Efficacies of Diazepam (DZP) and MK-801 as Treatments for Tetramethylenedisulfotetramine (TMDT) Poisoning in a Juvenile Rat Model

M. P. Shakarjian[2,3], M. Laukova[2], S. Shapiro[2], P. K. Stanton[2], J. Veliskova[2] and L. Velisek[2]. [1]*Cell Biology and Anatomy, New York Medical College, Valhalla, NY;* [2]*Environmental Health Science, New York Medical College, Valhalla, NY and* [3]*Medicine, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ.*

TMDT is a synthetic neurotoxic rodenticide identified in thousands of accidental, intentional, and mass poisonings in mainland China and worldwide. Symptoms include seizures which can escalate to staus epilepticus and lead to death of the victim. The agent is colorless, odorless, tasteless, water-soluble and easily synthesized, identifying it as a potentially desirable tool for terrorist use. Children have been exposed, and in some cases, intentionally targeted as victims of poisoning by this agent. Accordingly, we are examining the relative susceptibility of the juvenile population and their response to treatments, as compared to adults, in a rodent model. Postnatal day 15 (P15) rats were exposed to 0.2 mg/kg TMDT s.c. and treated, following the first significant symptom, with the GABA-A receptor positive modulator, DZP, or the NMDA receptor antagonist, MK-801, at various doses, i.p. We have previously found that P15 rats are more susceptible to TMDT than are adults, and that DZP and MK-801 are potentially beneficial treatments for the TMDT syndrome in adults. Our current studies show that these two agents can antagonize TMDT symptomatology in P15 rats and show near equal efficacy in inhibiting 24-hr lethality. However, DZP appears to be more effective than MK-801 in reducing both clonic and tonic-clonic seizures, and intrinsically more potent in juvenile rats than we have observed in adults. In conclusion, both GABA-A receptor positive modulators and NMDA receptor antagonists afford protection of juvenile rats from TMDT, but their level of efficacy may not be equivalent to that seen in adults. We are currently using EEG to confirm these results and examining whether there are sex differences in effectiveness of therapy. Supported by NIH R21NS084900.

1174 Neurotoxic Effects of Silver Nanoparticles on Developing Zebrafish (*Danio rerio*)

E. A. Gonzalez, M. La Merrill and G. Cherr. *Environmental Toxicology, University of California, Davis, Davis, CA.* Sponsor: M. La Merrill.

Silver nanoparticles (Ag-NPs) are frequently used materials because of their applications as antimicrobials. Their small size between 1–100 nanometers results in unique physiochemical properties that, despite their value, may also increase toxicity. As their usage and disposal can lead to release into aquatic environments, this study examines the neu-

rotoxic potential of these particles using developing zebrafish (*Danio rerio*) as a model system. Adult zebrafish were spawned and their embryos exposed to Ag-NPs from 24–72 hours post fertilization (hpf) at concentrations of 1, 3, 5, 7, 15, and 50 ppm (mg/L). A morphological assessment revealed significant spinal curvature and yolk sac edema (22.2%) beginning at 3 ppm Ag-NP. These occurrences rose to 28.8% in the 5 ppm Ag-NP treatment group. Due to high mortality beginning at 7 ppm, all subsequent experiments used 5 ppm as the concentration of interest. Qualitative Annexin V staining showed that 5 ppm Ag-NP treatments had visibly higher levels of fluorescence in the brain and head region than controls, indicating that Ag-NPs can induce apoptosis in the brain. A behavioral assessment suggested that even when no initial toxicity is observed in the form of morphological abnormalities, Ag-NPs may still cause detrimental health effects. Overall, this study demonstrates the wide range of toxic effects on development and the nervous system that are associated with Ag-NPs.

PS 1175 Effects of Vitamin D Receptor-Acting Compounds on Larval Motility in Zebrafish

A. N. Oliveri[1], D. Mahapatra[2], S. W. Kullman[2] and E. D. Levin[1]. *[1]Duke University Medical Center, Durham, NC and [2]North Carolina State University, Raleigh, NC.*

Vitamin D is important for a wide variety developmental processes in addition to ion homeostasis and bone growth. Critical among these roles is proper neurodevelopment. Toxicants that disrupt vitamin D signaling during brain development can produce neurobehavioral impairment. In this study we used the zebrafish model to provide an initial characterization of chemicals that act on the vitamin D receptor (VDR) during neurodevelopment and their effects on neurobehavioral function. Using a larval motility assay, the swimming activity of five-day old larvae was assessed by computer video analysis (DanioVision, Noldus, Inc.) in 96-well plates under alternating conditions of dark and light over a 50-min session in 10-min periods. Activity was recorded as mm/min. Testing took place on day five post-fertilization after treatment via immersion to vitamin D (0.5 and 2 nM) and select VDR antagonists PS121912 (2.5 and 5 μM), KT112213 (2, 5 and 10 μM), K-dicyanoaurate, cadmium HCl, cadmium dinitrate (5 and 10 μM each), and 0.1% DMSO control. There were 13-48 subjects per treatment condition and 87 controls. Increased lethality was seen with 5 and 10 μM KT112213 as well as 5 and 10 μM K-dicyanoaurate. Increased dysmorphogenesis was seen with 1 and 2 nM vitamin D, 2 and 5 μM KT112213, 2 and 5 μM K-dicyanoaurate and 10 μM cadmium HCl. Significant larval locomotor hyperactivity was seen with 2 nM vitamin D, 5 μM PS121912 (in the dark phase), 10 μM cadmium HCl, and 5μM cadmium dinitrate (in the dark phases). Locomotor hyperactivity was not consistently related to dysmorphogenesis, and even with the vitamin D- and cadmium HCl-exposed larvae, it appears unlikely that malformations would themselves cause hyperactivity. In contrast, significant larval hypoactivity was seen with 5 μM KT112213, which may be related to increased dysmorphogenesis. Further research will focus on persistent neurobehavioral effects as well as molecular and cellular mechanisms. Supported by the US EPA Star grant 83554101.

PS 1176 The Anti-Rheumatic Drug, Leflunomide, Implicates the Aryl Hydrocarbon Receptor in Altered Dopaminergic Biology

C. J. Mattingly, A. Planchart and E. Cook. *Biological Sciences, NCSU, Raleigh, NC.*

Leflunomide is a drug commonly used to treat rheumatoid arthritis. Its therapeutic effects are attributed to its metabolite, teriflunomide, which inhibits an enzyme important in *de novo* pyrimidine biosynthesis and T cell function. Previous studies also showed that leflunomide interferes with neural crest cell function leading to the loss of melanin production, and activates the aryl hydrocarbon receptor (Ahr) pathway. Melanin and the neurotransmitter, dopamine, are derived from tyrosine; therefore, we hypothesized that leflunomide might have neurological effects. Our studies in zebrafish revealed that tyrosine hydroxylase, an enzyme used in the conversion of tyrosine to dopamine, is downregulated and aberrantly expressed in response to leflunomide exposure. These results were consistent with adverse behavioral responses observed in zebrafish larvae exposed to leflunomide, in which responses to cycles of light and dark stimuli were significantly dampened compared to unexposed controls. Behavioral analyses in adult zebrafish exposed to low levels of leflunomide (500nM) showed increased anxiety as measured in a novel tank assay compared to unexposed controls, suggesting that the leflunomide effect is not ameliorated by a functional blood-brain barrier. In addition, behavioral changes were not observed in ahR2-null zebrafish, thus suggesting that leflunomide may affect neurological development via the Ahr signaling pathway.

PS 1177 Lipopolysaccharide-Induced Premature Delivery in Mice Is Accompanied by Disturbances in Central Respiratory Rhythm Generation

C. V. Smith, S. C. Ramirez, J. E. Koschnitzky, T. M. Anderson, N. A. Baertsch and J.-M. Ramirez. *Pediatrics, Seattle Children's Research Institute, Seattle, WA.*

Globally, almost one million premature infants die annually from respiratory insufficiency. In addition to developmental immaturity of their lungs, structural and central neonatal apneas contribute significantly, but have received far less attention. The present study tests the hypothesis that an animal model of premature delivery, administration of lipopolysaccharide (LPS) to pregnant mice, might produce newborn pups that exhibit traits of neonatal apnea in premature infants. This model parallels the human condition in that many premature human births are associated with chorioamnionitis and other types of maternal infections. Administration of 5 micrograms of LPS to C57BL/6 mice at day of gestation (G17.5) resulted in delivery of pups at G18.5, about half of which were viable for at least 2 h post delivery. In a flow, whole-body plethysmograph, premature mice from LPS-treated dams showed irregular breathing patterns, which in some pups transitioned to more mature patterns. Transverse, rhythmically active slices containing the pre-Bötzinger complex, the putative respiratory rhythm generator, obtained within 30 min after birth from term and C-sectioned pups exhibited rhythmic respiratory activity with regular frequency and amplitude. By contrast, slices from pups of LPS-treated dams generated respiratory rhythmic activities that were characterized by large scatters of amplitude and frequency, as well as irregular rhythmic activities. The respiratory activities in transverse slices correlated with the breathing patterns of individual mice *in vivo*. Pups that showed long-lasting, large amplitude breaths, gasps, and/or irregular breathing often showed large amplitude bursts and dissociated respiratory rhythmic activities in the isolated preBötC. Pups that showed regular eupneic breathing exhibited regular respiratory rhythmic activities in isolated transverse slices. The results suggest that disordered breathing in LPS-induced premature mice is associated with significant disturbances in central respiratory rhythm generation. Determining the cellular and molecular mechanisms that underlie disordered maturation of respiratory rhythm generation as contributors to disordered breathing in prematurely born mice is needed to provide a rational foundation for prevention and treatment of neonatal apnea in human infants.

PS 1178 Investigating a Mechanism of Developmental Neurotoxicity: Quaternary Ammonium Compounds and Their Effect on Cholesterol Biosynthesis

J. Herron[2], R. Reese[1], K. Mirnics[1], N. A. Porter[1] and L. Xu[2]. *[1]Department of Chemistry, Vanderbilt University, Nashville, TN and [2]Department of Environmental and Occupational Health Sciences and Department of Medicinal Chemistry, University of Washington, Seattle, WA; [3]Department of Medicinal Chemistry, University of Washington, Seattle, WA; [4]Department of Psychiatry, Vanderbilt University, Nashville, TN.*

Quaternary ammonium compounds (QAC) have been used in cleaning products, medical products, and the food processing industries, suggesting human exposure to QAC may occur through direct dermal contact, inhalation, and ingestion. Cholesterol plays important roles in embryonic development, synapse formation and function, and myelination. The cholesterol biosynthesis disorder, Smith-Lemli-Opitz syndrome, is caused by defective 3β-hydroxysterol-Δ7-reductase (DHCR7) and manifests into congenital malformations, neurodevelopmental delay, and autism-like behavior. Using an *in silico* approach, we found that benzalkonium chloride (BAC), the most common QAC, exhibits high structure similarity to AY9944, a known inhibitor of DHCR7. Previously, BAC has been shown to cross the blood-brain barrier. Thus, we hypothesized that QAC can impair neurodevelopment through the inhibition of cholesterol biosynthesis, leading to the accumulation of cholesterol precursors and oxidative stress associated with the highly oxidizable precursors. Utilizing cell viability assays, gas chromatography-mass spectrometry, real-time qPCR, and cell imaging, we investigated the effects of individual components of QAC on neuronal cells, including BAC with different side chains, didecyldimethylammonium chloride, cetrimonium chloride, and benzotrimethylammonium chloride. Our results indicate that QAC have varying degrees of cytotoxicity, reflecting the amphiphilicity of their structures. Individual QAC, including BAC, inhibits cholesterol biosynthesis at nM concentrations, as indicated by the accumulation of 7-dehydrocholesterol and 8-dehydrocholesterol. Structure-activity analysis was carried out to rationalize the different potency of individual QACs. Finally, gene expression changes related to cholesterol homeostasis suggest a direct inhibition mechanism by QACs.

1179 An Extended One-Generation Reproduction and Developmental Neurotoxicity (DNT) Drinking Water Study (OECD 443) of Ethylene Dichloride (EDC) in Crl:CD(SD) Rats

J. S. Bus[3], J. P. Maurissen[4], J. H. Charlap[5], C. A. Picut[5], R. Collins[1], C. J. Bevan[2] and J. C. Lamb[3]. [1]*BioSTAT Consultants Inc, Portage, MI;* [2]*CJB Consulting LLC, Loveland, OH;* [3]*Exponent Inc., Alexandria, VA;* [4]*JPM NeuroTox LLC, Midland, MI and* [5]*WIL Research, Ashland, OH.*

EDC (1,2-dichloroethane), a widely-used chemical intermediate for production of chlorinated chemicals, was given at targeted doses of 0, 50, 150 and 300 mg/kg/day to male (M) and female (F) parental (F0) rats (27/sex/group) at 28 days prior to breeding and continued in offspring (F1) up to postnatal day (PND) 123 (10–20 pups/sex in each of 4 cohorts). Based on PBPK modeling, doses were selected to avoid saturation of EDC metabolism; due to palatability of EDC in drinking water, average premating doses were 0, 38, 88, 183 (M) and 0, 40, 95 and 182 (F) mg/kg/day in F0 rats and 0, 37, 97, 184 (M) and 0, 39, 93 and 169 (F) mg/kg/day in F1 rats. Mid- and high-dose premating M F0 body weights were decreased 5.3 and 9.4%; F F0 body weights decreased 6.6 and 7.7%. F F0 body weight gains were not altered during gestation and lactation. PND 123 M F1 body weights were decreased 3.4, 10.8 and 17.3%; F F1 weights significantly decreased 5.8% only in the high dose. EDC did not cause any changes in reproductive performance (standard indices, sperm quality, estrous cyclicity), F0/F1 plasma T4 or TSH levels, M F1 anogenital distance and nipple retention, or M/F F0/F1 organ histopathology. No effects were seen on M/F F1 auditory startle responses (PND 20 and 60), motor activity or in a functional observational battery (PND 65). At PND 21, no effects were observed in M/F F1 perfused brain weight, length or width, or microscopic changes in 14 neural tissues. At PND 78, brain weights were significantly decreased in M at the mid- and high-doses, but not in F; EDC did not alter M/F brain width and length, morphometric data (8 layers) or histopathology (38 neural tissues). The overall No Observed Adverse Effect Level (NOAEL) for reproduction, hormone-sensitive endpoints and DNT was the highest dose tested. Funded by HAP Task Force.

1180 Developmental Alcohol Exposure Reduces Experience-Dependent Plasticity in Adolescent Mice without Affecting Cortical Microglial Behavior or Dendritic Spine Dynamics

E. L. Wong[2], G. O. Sipe[2], C. E. Lamantia[2] and A. K. Majewska[2]. [1]*Environmental Medicine, University of Rochester Medical Center, Rochester, NY and* [2]*Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY.*

Fetal alcohol spectrum disorder (FASD) is the leading cause of life-long, non-heritable mental disability, but the mechanisms of altered neuronal function in this disorder are not well understood. To understand such mechanisms, we exposed mice to high binge levels of ethanol (EtOH) during the human third trimester equivalent and found a deficit in experience-dependent synaptic plasticity in adolescence. When one eyelid is closed during the visual critical period (postnatal day 28 peak in mice), visual cortex neurons normally undergo plasticity, responding more to input from the open eye. While this ocular dominance plasticity (ODP) occurs in saline controls, it is less robust in mice previously exposed to EtOH. We investigated whether changes in the neuro-immune interface underlie the observed reduction in ODP. Specifically, we used *in vivo* two-photon microscopy to dynamically image dendritic spines, microglia, and their interactions in the intact, living, adolescent mouse cortex. Dendritic spines are tiny protrusions on neuronal branches that represent the postsynaptic sites of most excitatory synapses. Microglia are the brain's resident immune cells known for their acute response to infection, injury, or environmental insult, including high binge levels of EtOH. Outside of pathology, microglia have highly ramified processes that contact dendritic spines, influencing synaptic architecture during plastic events. ODP is known to rely on dynamic remodeling of dendritic spines. Therefore, we hypothesized that developmental EtOH exposure would result in abnormal dendritic spine turnover and microglial behavior in adolescent mice. We found that EtOH had no effect on the basal turnover rate of dendritic spines. Microglial process motility and the response of microglia to focal injury were also not altered by prior EtOH exposure. Analysis of dendritic spine density, morphology, and microglial-spine contact is in progress, but no overt EtOH-induced differences have been noted. Thus, the observed functional deficit in experience-dependent plasticity is likely not the result of long-term changes in baseline dendritic spine dynamics or in the physiological or pathological behaviors of cortical microglia.

1181 High-Taurine Consumption During Adolescence Alters Biogenic Amines in a Sex-Dependent Manner in C57BL/6J Mice

J. Weimer, J. Brown, C. Ludwig and C. P. Curran. *Biological Sciences, Northern Kentucky University, Highland Heights, KY.*

Energy drinks are a multi-billion dollar industry, and more than half of all energy drinks are consumed by adolescents and young adults. A key ingredient in the top-selling energy drinks is the amino acid taurine. Taurine naturally exists as the most abundant free amino acid in the central nervous system of mammals. Although taurine can be neuroprotective and act as an antioxidant, recent studies indicate there is a risk of neurotoxicity at high doses. To determine if excess taurine could damage the maturing brain, we treated adolescent C57BL/6J mice with distilled water or water containing 0.12% taurine (400 mg/kg) from postnatal day 30 to 60 when behavioral experiments were conducted. We previously reported that taurine-treated mice showed deficits in Novel Object Recognition and that taurine-treated males showed deficits in Morris Water Maze. We also found that taurine-treated males had an enhanced response to caffeine. To further probe these differences, we compared neurotransmitters in three brain regions: the cerebellum, cortex and hypothalamus. We found a significant difference in serotonin levels ($P < 0.05$) in the hypothalamus between taurine-treated and control mice and a trend for a treatment x sex interaction ($P = 0.094$) for levels of the serotonin metabolite 5-HIAA. In the cortex, there was a trend for a treatment x sex interaction ($P= 0.072$) for dopamine levels with taurine-treated males showing decreased dopamine whereas taurine-treated females had higher levels than controls. In the cerebellum, there was a significant main effect of sex ($P < 0.01$) and a significant treatment x sex interaction ($P < 0.01$) for levels of the serotonin metabolite HVA. Taurine-treated females had lower levels than controls whereas males had higher levels than controls. Together, these data suggest that high taurine consumption during adolescence alters biogenic amines in multiple brain regions in a sex-dependent manner which could explain some of the behavioral deficits previously observed. Supported by ES020053 and GM103436.

1182 The Effects of DEHP on Placenta and Fetal Brain Development

Y.-C. Chuang[2], J.-Y. Chiu[2], M.-W. Chao[2] and C.-Y. Tseng[2]. [1]*Department of Biomedical Engineering, Chung Yuan Christian University, Taoyuan, Taiwan and* [2]*Department of Bioscience Technology, Chung Yuan Christian University, Taoyuan, Taiwan.*

DEHP is the most abundant phthalate plasticizer in polyvinyl chloride formulations, including vinyl flooring, wall covering, food containers, gloves, and infant toys. It can be leached into a liquid that comes in contact with plastics, suggesting concerns about the health risk of human exposure. Previous animal studies have suggested that maternal exposure to phthalates may cause reproductive and developmental toxicities in offspring. Other studies have identified that maternal-placental-fetal interaction can influence brain development. We hypothesize that DEHP is harmful to fetal brain in development by affecting the configuration of placenta. DEHP (125 mg/kg/day) was given to pregnant Sprague-Dawley rats orally from the second day of gestation (GD 2) to GD 18, and the rats were sacrificed at GD 19. The section of placentas and fetal brains were collected and analyzed with hematoxylin and eosin, or immunochemistry. We found that there was no difference in the placental shape and weight between control and the DEHP-treated samples. However, the thickness in labyrinth was decreased, and the one of spongiotrophoblast area was increased. The increase of widths in labyrinthine vessels lead to labyrinthine morphological changes and vasculature disorganization in DEHP-treated group. To asses the effects of DEHP on neurogenesis, bromodeoxuridine (BrdU) was injected in three continuative days from GD 16, and the layer-specific markers, Tbr1 (layer VI), Ctip2 (layer V) and Satb2 (layer II/III) were used to determine the cortical laminae patterning. Our data shows an abnormal layer organization in developmental fetal brain. In addition, DEHP induced ROS generation, which disrupts the placental, and fetal brain organization and its development. This study suggests that maternal exposure of DEHP influence placenta configuration and fetal brain development because of DEHP induced oxidative stress.

1183 Hypoxic Preconditioning Modulates Anti-Inflammatory Responses Mediated by Disrupted in Schizophrenia-1(disc1) Gene Expression

J.-R. Liu, X. Han, Y.-J. Li and J. Ibla. *Anesthesia, Harvard Medical School, Boston, MA.*

Background: Disrupted in Schizophrenia 1 (DISC1), as a potential susceptibility gene for schizophrenia, was identified as a result of (1; 1) (q42; q14.3) translocation in a Scottish family afflicted by severe mood disorders. In our previous study, suppression of NF-kB activity has demonstrated to be a critical anti-inflammatory feature induced by Hypoxa and re-oxygenation (H/R). Recently, we found that DISC1 could affect NF-kB activity. Thus, we studied the role of DISC1 gene expression as genetic factor involved in the regulation of NF-kB activity mediated by ataxia telangiectasia mutated (ATM) and induced by H/R. Methods: *In vivo* and *in vitro* models of DISC1 exogenous over-expression and knock-down were exposed to protocols of H/R and environmental hypoxia. The patterns of ATM phosphorylation and cellular co-localization were studied by western blot and Immunofluorescence. Gene expression and secretion of IL-6 were quantified in cells expressing high and low levels of DISC1 by Real-Time PCR and ELISA. Results: We identified a significant inhibitory effect on ATM-mediated NF-kB activation cascade mediated by DISC1. We demonstrate that DISC1 over-expression both significantly attenuate hypoxia-mediated ATM phosphorylation and prevent its cytoplasm translocation where it functions to activate NF-kB. We established that DISC1 cellular levels modulate Protein Phosphatase 2A (PP2A) activity, specifically controlling the phosphorylation of ATM Serine-1981, the main regulatory site of ATM activity. Epithelial cell levels of DISC1 protein significantly modulate NF-kB activation profiles and pro-inflammatory gene expression (IL-6). Conclusions: H/R induces the expression of DISC1, modulating ATM activation via PP2A and NF-kB-regulated gene expression. Together these results implicate DISC1 in NF-kB signaling and provide novel basis for understanding inducible anti-inflammatory responses mediated by H/R.

1184 Novel Human Hindbrain and Spinal Cord Model for Neurotoxicity Studies

N. Sehgal and R. Ashton. *Wisconsin Institute of Discovery, University of Madison, Madison, WI.* Sponsor: T. Knudsen.

Translation of drug discovery and toxicological screening results to human biology is often questionable due to the lack of reliable model systems. In particular, significant anatomical and physiological discrepancies exist between human and rodent central nervous systems (CNS), and developing models that recapitulate the phenotypic diversity evident *in vivo* at regional, cellular and molecular levels is very challenging. However, human pluripotent stem cells (hPSCs) have emerged as a viable source of all CNS cell phenotypes, which can be used to study neurodevelopment, patient-specific neurological disorders, and neurotoxicity. Our lab has developed chemically defined, reproducible methods for differentiating hPSCs into neural stem cells of both forebrain (Lippmann et al. Stem Cells 2014) and discrete hindbrain thru spinal cord rostrocaudal regions (Lippmann et al. Stem Cell Reports 2015). From this spectrum of NSCs, we now present a comprehensive, *in vitro* model of the hindbrain and spinal cord containing neuronal tissues from diverse rostrocaudal (R/C) and dorsoventral (D/V) coordinates. For example, the model is comprised of diverse ventral motor neurons subtypes and pools and dorsal interneurons populations thereby capturing critical facets of cell phenotype diversity in the human CNS. Furthermore, temporal RNA-seq analysis is used to map the evolution of phenotypical diversity within the model, and provide a distinct gene expression signatures for each tissue. This provides a novel tool for human neurotoxicology that enables rapid screening of cell phenotype-specific toxicity through comparative RNA-seq analyses

1185 Embryonic 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Exposure Disrupts Central Nervous System (CNS) Angiogenesis and Blood Brain Barrier (BBB) Development

J. S. Plavicki, M. S. Yue, T. R. Baker, M. R. Taylor and R. E. Peterson. *Pharmaceutical Sciences, University of Wisconsin, Madison, WI.*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant that exerts toxicity by activating aryl hydrocarbon receptor (AHR) signaling. AHR signaling is necessary for proper vascular development during mammalian organogenesis. In zebrafish, TCDD-induced Ahr2 activation has been reported to produce two cerebrovascular malformations as well as increased albumin permeability in the dorsal midbrain. However, it is not known whether exogenous Ahr2 activation during embryogenesis produces additional cerebrovascular malformations or globally disrupts BBB development in the zebrafish brain. To examine in greater detail how global Ahr2 activation disrupts CNS angiogenesis, we exposed zebrafish from the endothelial reporter line, kdrl:EGFP, to the prototypical AHR ligand, TCDD (1ng/ml; waterborne). TCDD exposure inhibited central artery development in the fore-, mid-, and hindbrain. Using confocal time-lapse experiments, we discovered that Ahr2 activation altered brain endothelial cell (BEC) behavior and resulted in aberrant vessel morphology and impaired lumen formation. We are using the kdrl promoter to drive endothelial expression of a constitutively active AHR (caAHR) or, as a control, a caAHR that is unable to bind DNA (caAHR-dbd). Preliminary studies indicate that endothelial-specific expression of caAHR is sufficient to produce hemorrhaging and disrupt central vervous system (CNS) angiogenesis. To test whether global Ahr2 activation disrupts blood brain barrier (BBB) development, we exposed transgenic embryos carrying a marker of differentiated BECs (glut1b) to TCDD. Global Ahr2 activation inhibited glut1b in early CNS angiogenesis and continued to inhibit glut1b expression as development progressed. In zebrafish, angiogenesis and barriergenesis occur simultaneously. The lack of glut1b expression indicates that the endothelial cells have not differentiated properly or acquired barrier properties. Together, our results indicate that AHR activation impairs both CNS angiogenesis and BBB development. (Supported by NIH K99 ES023848).

1186 Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (tcdd) Induces Expression of Prostaglandin E Synthase 3 and N-methyl-d-aspartate-receptor 1 Splice Variants 3 and 4 in the Developing Anteroventral Periventricular Nucleus (avpv)

J. Del Pino[1] and S. L. Petersen[2]. [1]*Toxicology and Pharmacology, Complutense University of Madrid, Madrid, Spain and* [2]*Veterinary and Animal Sciences, University of Massachusetts, Madrid, MA.*

Developmental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) interferes with masculinization of gonadotropin release patterns, most likely by blocking estradiol (E2)-dependent sexual differentiation of the anteroventral periventricular nucleus (AVPV). The process of sexual differentiation involves both cell death and alterations in neurite outgrowth, but the underlying molecular mechanisms are unclear. It has been reported that dendritic spine number and shape is modified by TCDD. PGE2, which is synthetized by prostaglandin E synthase (PTGESs), induces formation of dendritic spines on preoptic area (POA) neurons through EP receptor interaction. We identified CUG triple repeat RNA binding protein 2 (CUGBP2) as a sex-specific gene significantly upregulated by E2 in females and downregulated by TCDD in males on PND 2. Cugbp2 regulates expression of PTGES 2 enzyme and expression and splicing of the N-methyl-d-aspartate-receptor 1 (NR1) subunit of NMDA receptor. Furthermore, we reported previously that TCDD increased NR1 expression and glutamate signaling through the NMDA receptor. Moreover, NR1 splice variants have been implicated in dendritic outgrowth and synaptogenesis in other brain regions, but no work has examined whether if PTGESs enzymes or NR1 splice variants are affected by TCDD in the AVPV.To determine whether these PTGESs enzymes and NR1 variants were altered by TCDD in the AVPV, we administered vehicle or TCDD 600 ng orally to rat dams on the first post-partum day. Pups were euthanized the following day (PND2) and AVPV regions microdissected from frozen sections. Using QPCR, We found that mRNAs encoding PTGES 3 and NR1 variants 3 and 4 were differently expressed between the sexes and were significantly upregulated by TCDD in males. These findings suggest that TCDD may interfere with masculinization of the AVPV by increasing PGE2 and EP receptor levels, as well as by controlling NR1 mRNA synthesis and splicing.

1187 Assessing the Role of the Transcription Factor Sox9b in TCDD-Induced Cerebrovascular Malformations

J. C. Gawdzik[2], M. R. Taylor[2], R. E. Peterson[2] and J. S. Plavicki[2]. [1]*Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI and* [2]*Pharmaceutical Science, University of Wisconsin, Madison, WI.*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a toxic, environmentally persistent, and ubiquitous compound. Zebrafish embryos exposed to TCDD (1ng/mL waterborne) shortly after fertilization have cerebrovascular malformations, cerebral edema, and hemorrhaging. Preliminary data indicate that TCDD exposure reduces expression of the SRY-box 9b (sox9b) gene in the central nervous system of developing zebrafish. We hypothesized that globally reducing the level and function of Sox9b would recapitulate the malformations seen in the zebrafish cerebral vasculature following TCDD exposure. To visualize and assess the cerebral vasculature following sox9b knockdown, we injected sox9b morpholinos (MO) into a transgenic zebrafish endothelial cell reporter line

(kdrl:EGFP). At 48 and 72 hours post-fertilization (hpf), kdrl:EGFP sox9b morphants displayed cerebrovascular malformations reminiscent of those observed following early embryonic TCDD exposure. In addition, we generated a dominant-negative Sox9b (dnSox9b) variant lacking the C-terminal transactivation domain that blocks transcription of Sox9b target genes both *in vitro* and *in vivo*. Consistent with the sox9b MO phenotypes, injecting dnsox9b RNA into another zebrafish endothelial cell reporter line (fli:EGFP) impaired cerebrovascular development at 72 and 96 hpf. Since Sox9b is expressed in multiple cell types in the developing central nervous system, we are generating transgenic zebrafish lines to manipulate Sox9b function in a cell-type specific manner. This will enable us to determine in which cell-types loss of Sox9b function results in cerebrovascular phenotypes. Supported by NIH T32 ES007015 (JCG), AFPE (JCG) and NIH K99 ES023848 (JSP)

1188 Developmental Mismatch Between Fetal and Adult Steroid Concentrations: Enhanced Hormonal Dysregulation in Adult Mice Maternal Exposed to a Mixture of Low Dose Endocrine Disrupting Chemicals

M. Sobolewski, K. Conrad, K. Harvey, K. Morris-Schaffer, C. R. Klocke, J. L. Allen and D. A. Cory-Slechta. *University of Rochester Medical Center, Rochester, NY.*

Understanding the toxicity of low dose, endocrine disrupting chemical (EDC) mixtures is of paramount importance given that humans are exposed to mixtures of chemicals, and previous studies demonstrate that mixtures of differentially-acting androgens can produce additive male reproductive dysfunction, but little is currently known about CNS effects. To assess the hormonal consequences of developmental EDC mixture exposure, pregnant female mice were exposed to near estimated reference level doses of four EDCs: Atrazine (ATR: 10mg/kg), Perfluorooctanoic acid (PFOA: 0.1 mg/kg), Bisphenol-A (BPA: 50µg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD: 0.035µg/kg) and their mixture (MIX) or to vehicle, daily from gestational day 7 until weaning. Our previous research identified unique, sex-specific behavioral deficits in attention, fear and sociality in males, following mixture exposure. In addition, our data revealed male-specific increased testosterone (T) concentrations at birth with concomitant reproductive malformations. T is critically important for sexual differentiation of the brain and reproductive organs, as well as the conservation of sexually-dimorphic behavioral patterns. To assess the lifelong consequences of this EDC mixture induced increase in T, peripheral steroid hormone concentrations in adulthood were measured using ELISA assays for serum T and corticosterone. In contrast to the increased T found at birth during the exposure period, T levels in adulthood, long after exposure ceased, were significantly decreased in MIX exposed males. In accordance, corticosterone levels were also reduced in MIX males, however, slight reductions were seen in PFOA exposed males. There were no significant alterations in female steroid levels. These data provoke questions on the fetal origins of endocrine programming into adulthood and the categorization of EDC action as anti-androgenic or androgenic. The sex-specific nature of these deficits provides fundamental insight into the sexually-dimorphic behavioral deficits observed, which is particularly relevant as inappropriate levels of perinatal T have been posited to play an etiological role in the development of children's male-biased neurobehavioral diseases. P30 ES001247 & T32 ES007026-38.

1189 A Pilot Study on Possible Involvement of Dioxin Exposure in Neurodegenerative Diseases

Y. Chen, T. Xu, S. L. Xu, H. Q. Xie and B. Zhao. *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Chinese Academy of Sciences, Beijing, China.*

Cognition reflects multiple and advanced functions of cerebral cortex. Cognitive decline is associated with several neurodegenerative diseases, such as Alzheimer`s disease (AD). Many environmental pollutants could cause structural and functional injuries in the cerebral cortex, and contribute to cognitive deficit through different mechanisms. Exposure to dioxin and dioxin-like compound in early life negatively influenced cognitive ability of middle-aged and aged people by epidemiology studies. Cognitive defect in dioxin-exposed animals also suggested the possible correlation of dioxin exposure with higher risk of AD and other neurodegenerative diseases. However, biological plausibility underneath this correlation is not fully understood. In this study, we identified genes induced by dioxin or regulated by aryl hydrocarbon receptor signal pathway in the neuronal system from microarray database (GEO datasets) and our own laboratory database (unpublished experimental data). Gene ontology and KEGG pathway enrichment analysis were applied to predict in which pathways and biological processes these genes might be involved. Altered genes related to neurodegenerative diseases, the potential signal pathway and biological processes were predicted by the same methods. Overlapping genes were found while comparing the two groups of interested genes. By pathway and functional clustering as well as literature review, these overlapping genes were found to take part in several generally accepted common mechanisms of different neurodegenerative diseases, such as protein aggregation, mitochondrial dysfunction, oxidative stress, inflammation, brain-blood barrier disruption, and malfunction of some typical molecular biomarkers in the pathogenesis and progression of the diseases. Thus, we proposed a possibility that bio-interferences of dioxin and dioxin-like compound might be involved in the onset or progression of neurodegenerative diseases. More detailed mechanism study is demanded to further reveal the relationship between dioxin and neurodegenerative diseases.

1190 Constitutive Activation of Ahr Signaling Disrupts Dendritic Growth and Cellular Migration of Cortical Pyramidal Neurons in the Developing Mouse Brain

E. Kimura[2], K.-i. Kubo[1], T. Endo[2], W. Ling[2], K. Nakajima[1], M. Kakeyama[2,3] and C. Tohyama[2,4]. *[1]Department of Anatomy, Keio University, Tokyo, Japan; [2]Department of Medicine, The University of Tokyo, Tokyo, Japan; [3]Faculty of Human Sciences, Waseda University, Saitama, Japan and [4]Faculty of Medicine, University of Tsukuba, Ibaraki, Japan.*

In utero and/or lactational exposure to dioxins has been shown to impair higher brain function in both humans and laboratory animals, presumably via the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor known to mediate a variety of dioxin toxicities. Dendritic growth and the cellular migration of immature neurons in the developing brain are prerequisites for the normal development of the neural network to ensure higher brain function. In the present study, we investigated whether and how the constitutive activation of AhR signaling affected the neural network structure in terms of dendritic growth and cellular migration in the developing mouse cerebral cortex. Pregnant C57BL/6 mice were subjected to *in utero* electroporation under anesthesia on gestational day 14.5, and the expression vectors of EGFP, EGFP + AhR, or EGFP constitutively active (CA)-AhR were electroporated into the lateral ventricle of the intrauterine embryos. On postnatal day 14, the mice that underwent *in utero* electroporation were anesthetized and perfused with 4% PFA. Isolated brains were cut through the coronal plane to obtain 150-µm sections of the cerebral cortex. Dendritic morphology and cellular distribution were analyzed using Neurolucida neuron tracing software and ImageJ software, respectively, and compared between the groups. In the developing cerebral cortex, the dendritic complexity and the entire length of EGFP-labeled pyramidal neurons were significantly decreased in the CA-AhR group compared with the control and AhR groups. Next, we examined the effect of CA-AhR on the cellular migration of cortical pyramidal neurons and found a delay in cellular migration in the CA-AhR group compared with the control group. These histological abnormalities in the developing brain are thought to induce the impairment of higher brain function. In conclusion, our findings showed that AhR signaling activation disrupts the development of the neural network by abnormal dendritic growth and cellular migration, suggesting the presence of a common mechanism of developmental neurotoxicity in response to perinatal dioxin exposure in mice.

1191 PCB 95-Induced Dendritic Growth in Primary Cultures of Rat Hippocampal Neurons Is Dependent on mTOR Activation

G. W. Miller, K. Keil, H. Chen, K. Dhakal, S. Sethi, J. W. Kim and P. J. Lein. *Department of Molecular Biosciences, University of California Davis, Davis, CA.*

Despite being banned since the late 1970s, polychlorinated biphenyls (PCBs) remain persistent environmental toxicants that pose significant risk to the developing nervous system. We recently demonstrated that the non-dioxin-like PCB 95 alters neuronal connectivity by promoting dendritic arborization in cultured hippocampal neurons via ryanodine receptor (RyR)-mediated, transcriptionally driven mechanisms. PCB 95 sensitizes RyR and this interaction requires FKBP12. Interestingly, FKBP12 also regulates mechanistic target of rapamycin (mTOR), and the mTOR signaling pathway enhances dendritic growth via translational mechanisms. Based on these observations, we hypothesize that mTOR signaling contributes to the dendrite promoting activity of PCB 95. To test this hypothesis, primary cell cultures dissociated from hippocampi of Sprague Dawley postnatal rats were plated at high density and exposed to PCB 95 (2,2'3,5'6-pentachlorobiphenyl, 20 pM - 2 nM) in the absence or presence of mTOR modulators. Exposure to PCB 95 from days 7 to 9 *in vitro* increased dendritic growth in pyramidal hippocampal

neurons in a concentration-dependent manner. Simultaneous exposure to rapamycin (20 nM) or FK-506 (0.5 - 50nM) at concentrations that inhibit mTOR activation inhibited the dendrite promoting activity of PCB 95. ELISA analyses for levels of phospho-specific signaling components downstream of mTOR confirmed that PCB 95 activated mTOR signaling. Furthermore, knockdown of mTOR using siRNA prevented PCB 95-induced dendritic growth. These findings identify a novel mechanism by which low-level exposures to RyR-active PCBs cause developmental neurotoxicity, and perhaps dysfunction of other cell types, such as immune cells, whose function is heavily dependent on mTOR signaling. This work supported by the NIEHS (grant ES014901 to PJL and postdoctoral fellowship ES024070 to GWM).

1192 DNA Methylation as a Mediator of 2,2′,3,5′,6-Hexachlorobiphenyl (PCB 95) Induced Dendritic Arborization

K. P. Keil, S. Sethi and P. J. Lein. *Molecular Biosciences, University of California Davis, Davis, CA.*

Environmental exposures are implicated in the pathogenesis of many neurodevelopmental disorders but the mechanisms by which they act to alter neurodevelopment remain speculative. Since altered dendritic arborization is associated with many neurodevelopmental disorders, we tested the hypothesis that DNA methyltransferases (DNMTs) mediate the enhanced dendritic arborization observed in central neurons following *in vivo* or *in vitro* exposure to the environmental neurotoxicant, polychlorinated biphenyl (PCB) 95. Neuron-glia co-cultures dissociated from hippocampi and cortices isolated from male and female C57BL/6 mice or Sprague Dawley rats at postnatal day 0 were treated from days *in vitro* 7–9 with one or more of the following: vehicle (0.1% DMSO), PCB 95 (1pM-1μM), pan-DNMT inhibitor RG108 or siRNA targeting Dnmt3b. Dendritic morphology and relative DNMT abundance were quantified using Sholl analysis, immunocytochemistry and MetaMorph image analysis software. Treatment with RG108 alone increases dendritic arborization in female mouse cortical neurons and enhances the dendrite promoting activity of PCB 95 in female hippocampal and cortical neurons. Knockdown of DNMT3B using Dnmt3b siRNA phenocopies the effects of PCB 95 on dendritic growth. Exposure to PCB 95 decreases the ratio of nuclear/cytoplasmic intensity of *de novo* DNMTs 3A and 3B but not the maintenance DNMT1 relative to vehicle control in rat hippocampal neurons, implicating *de novo* DNMTs in regulation of dendritic growth. Collectively, these results suggest that DNMTs regulate basal dendritic growth *in vitro* and that DNMTs mediate, at least in part, PCB 95 effects on dendritic growth. These observations suggest a novel mechanism of PCB developmental neurotoxicity that may represent a convergence point for gene-environment interactions that influence the risk and/or severity of neurodevelopmental disorders. Funding: NIH ES014901, ES011269 and US EPA R833292.

1193 3,3′-Dichlorobiphenyl (PCB 11) and Its Metabolites Increase Dendritic Arborization in Primary Rat Cortical and Hippocampal Neurons

S. Sethi[1], K. Keil[1], K. Hayakawa[1], H. Chen[1], F. Wei[1], Y. Dong[1], X. Li[2], I. Pessah[1], H.-J. Lehmler[2] and P. Lein[1]. [1]*Molecular Biosciences, University of California Davis, Davis, CA and* [2]*Occupational & Environmental Health, University of Iowa, Iowa City, IA.*

PCB 11 has recently been identified as a byproduct of paint pigment production and a ubiquitous air pollutant in multiple regions of the United States. Whether human exposure to PCB 11 is a public health concern is unclear since few studies have evaluated its toxicity. Neurotoxicity is a primary endpoint of concern for the more highly chlorinated PCBs, therefore, this study investigated the effects of PCB 11 and two of its metabolites on dendritic arborization in primary neuron-glia co-cultures dissociated from the neocortices or hippocampi of neonatal Sprague Dawley rats. At 6 days *in vitro* (DIV), co-cultures were transfected with a MAP2B-eGFP construct to label the dendritic arbors of individual neurons; at 7 DIV, cultures were treated for 48 h with PCB 11, its 4-OH or 4-OSO3- metabolite or vehicle (0.1% DMSO). Dendritic morphology was quantified in GFP-labeled neurons by Sholl analysis. Exposure to PCB 11 or its metabolites at picomolar concentrations increased dendritic arborization with more potent effects observed in cortical relative to hippocampal neurons. We next tested whether the ryanodine receptor (RyR) and the aryl hydrocarbon receptor (AhR) mediated the dendritic promoting activity of PCB 11. [3H]Ryanodine binding assays failed to indicate that PCB 11 directly modulates RyR1 or RyR2 conformation and pharmacologic antagonism of the RyR or AhR had minimal effect on PCB 11-induced dendritic growth. Since altered patterns of dendritic arborization are associated with neurodevelopmental disorders, these findings suggest that PCB 11 may pose a potential risk to the developing human brain via mechanism(s) not previously described for the more highly chlorinated PCB congeners. Supported by a Floyd and Mary Schwall Predoctoral Fellowship to SS and NIEHS grants ES014901 to PJL and INP and ES013661 to HJL.

1194 Defining the Neurotoxic Potential of Nondioxin-Like PCBs Present in Fish from US Lakes Using a Ryanodine Receptor-Equivalency Scheme

E. B. Holland[1,2], W. Feng[2], Y. Dong[2] and I. N. Pessah[2]. [1]*Biological Sciences, CSU Long Beach, Long Beach, CA and* [2]*Molecular Biosciences, UC Davis, Davis, CA.*

Polychlorinated biphenyl (PCB) risk assessment is largely based on dioxin equivalents (TEQ) assessing the relative potency of 209 congeners at the aryl-hydrocarbon receptor (AhR) to predict organismal toxicity. Neurotoxic non-dioxin-like (NDL) PCBs have little to no activity towards AhR and their risk of promoting neurotoxicity is underestimated by current assessment schemes. Here, we aimed to establish a neurotoxic equivalency (NEQ) scheme based on PCB potency towards ryanodine receptor (RyR) Ca2+ channel activation, a sensitive target of NDL-PCB toxicity, as an important risk assessment tool. [3H]Ryanodine ([3H]Ry) binding analysis and single channel voltage clamp is used to measure the relative RyR activity of PCB congeners with two to eight chorine substitutions. Receptor based toxicity is compared to that predicted in a previous quantitative structure-activity relationship (QSAR) predicting the RyR-neurotoxicity of all 209 PCBs. RyR-based NEQs are applied to PCB concentrations found in fish from United States (US) lakes to estimate neurotoxic risk. The activity of individual PCB congeners follows the general pattern predicted by the developed QSAR. Tetra-ortho PCB202 was found to be the most potent congener at the RyR increasing channel open probability at just 200 pM. Notably, para-chlorines reduce the relative receptor activity of PCB congeners as compared to congeners with similar substitution patterns. When PCB202 based NEQs are applied to PCB concentrations found in fish from US lakes a conservative estimate of 20 or 48% of US lakes have predatory or bottom dwelling fish, respectively, containing significant PCB neurotoxic equivalents. NEQ based on RyR-sensitization combined with the established dioxin TEQ provides a more inclusive measure of risk presented by PCB mixtures.

1195 Genetic Susceptibility to Developmental Delays and Neurotoxicity in PCB-Treated Mice

K. Klinefelter[2], A. L. Juarez[1], S. Hampton[2], M. Kromme[2], M. Stegman[2] and C. P. Curran[2]. [1]*Cincinnati Children's Medical Center, Cincinnati, OH and* [2]*Biological Sciences, Northern Kentucky University, Highland Heights, KY.*

Polychlorinated biphenyls (PCBs) are neurotoxic pollutants found mainly in our food supply that cause motor impairments, learning and memory deficits, and are known to disrupt thyroid hormone levels. Previously, we reported that both high-affinity AhrbCyp1a2(-/-) and poor-affinity AhrdCyp1a2(-/-) are more susceptible to developmental PCB exposure compared with wild type AhrbCyp1a2(+/+) mice. Our current studies are aimed at looking at the underlying biochemical and gene expression changes responsible for the observed learning, memory, and motor deficits. We treat pregnant dams using corn oil-soaked food or food dosed with an environmentally relevant mixture of coplanar and noncoplanar PCBs. Treatments begin on gestational day 0 and continue until weaning at postnatal day 25 (P25). We used enzyme-linked immunoassays to measure thyroid hormone levels in plasma of mice at P14, based on previous studies showing that is a critical time for thyroid hormone disruption in early brain development. We found a significant main effect of treatment ($P < 0.05$) with PCB-treated mice having lower T4 levels than controls as well as reduced spleen ($P < 0.01$) and thymus ($P < 0.001$) wet weights. There was also a significant gene x treatment interaction with the most susceptible AhrbCyp1a2(-/-) having significantly reduced spleen ($P < 0.001$) and thymus ($P < 0.001$) wet weights. Our gene expression studies focused on P25 when PCB exposure is highest in pups. We confirmed CYP1A1 induction only in livers of the high-affinity Ahrb mice. In the cerebellum, we found down-regulation of neurotrophin3 in the cerebellum of high-affinity Ahrb mice, but no change in CYP1A1, BDNF, and myelin basic protein (MBP) in any PCB-treated mice. Together, these results confirm our previous results that both the AHR and CYP1A2 affect susceptibility to developmental PCB exposure and that thyroid hormone disruption is highest in mice with the Ahrb genotype. Supported by ES020053 and GM103436.

1196 Sustained Metabolic Phenotype and Associated Neuroimmune Effects After Prenatal Trichloroethylene Exposure

S. J. Blossom[1], S. B. Melnyk[1], W. D. Wessinger[1], M. Li[1] and C. Cooney[3]. [1]*Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR;* [2]*Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR and* [3]*Research and Development, Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Trichloroethylene (TCE) is an industrial solvent and environmental contaminant. There is a need to document health effects and to define windows of susceptibility for development of therapeutic interventions. We have studied immunotoxicity and neurotoxicity in a mouse model after developmental exposure including the postnatal period. Here we examined whether exclusively prenatal TCE exposure could alter behavioral, neural oxidative stress responses, and peripheral CD4+ T cell cytokine phenotype and function similar to that observed with other exposure periods. Three groups of breeder pairs were given water with 0, 0.01, and 0.1 mg/ml TCE (0, 3, and 29 mg/kg/day). At postnatal day 0, the TCE-containing water bottles were removed. Male offspring were examined at 6 weeks of age for locomotor and social approach behaviors using EthoVison video tracking system. Further analysis included glutathione and cysteine redox metabolites and oxidative stress biomarkers including 3-nitrotyrosine in cerebellum and plasma by HPLC. Phenotypic analysis of CD4+ T cells was carried out using CyFlowML flow cytometer. CD4+ T cell cytokines were determined by Luminex using Multiplex kits. TCE exposure increased locomotor activity in male offspring. Latency to approach the center of the arena was also significantly lower in TCE-treated mice. Social behaviors were not altered by prenatal TCE exposure. Regression analysis supported an inverse correlation with loss of anti-oxidant support (i.e., decreased cysteine) and increased locomotion ($r= -0.51$; $p=0.02$). Oxidative stress endpoints were increased in plasma and cerebellum. Prenatal TCE exposure increased the population of effector memory CD4+ T cells and enhanced IFN-γ and IL-17 levels. CD4+ T cell production of IFN-γ was positively correlated with oxidative endpoints in TCE-treated mice ($r=0.84$; $p=0.03$). The results demonstrated redox imbalance, oxidative stress, increased locomotor activity and T cell responses are linked and sustained in adult mice with prenatal exposure. These data may provide important clues to the etiology of several neurodevelopmental disorders believed to originate *in utero*.

1197 BDE-47 and BDE-49 Inhibition of Axon Outgrowth in Cultured Hippocampal Neurons Is Reversed with Triiodothyronine or Antioxidant Co-Treatment

H. Chen, A. C. Bautista, K. Hayakawa and P. J. Lein. *Molecular Biosciences, University of California Davis, Davis, CA.*

Polybrominated diphenyl ethers (PDBEs) are a class of brominated flame retardants that were widely used in textiles, polyurethane foams, and plastics. Higher body burdens of PDBEs in children are associated with increased risk of cognitive deficits, including decreased attention and performance on intelligence tests. However, the molecular and cellular mechanisms associated with PBDE developmental neurotoxicity are not clearly understood. PBDEs are recognized endocrine disrupters and exposure has been linked to decreased levels of serum thyroid hormone (TH). Additionally, PBDE exposure has been linked to oxidative stress and increased reactive oxygen species production. Neuritogenesis is sensitive to changes in TH and redox signaling, and changes in neurite outgrowth are associated with neurodevelopmental disorders. To test the hypothesis that PBDEs modulate neurite outgrowth via effects on TH and/or redox signaling, we quantified axonal length in primary dissociated hippocampal cultures exposed to BDE-47 or BDE-49 at 200 nM from days *in vitro* 0–2, in the absence or presence of either triiodothyronine (T3, 3 nM) or N-acetyl cysteine (NAC, 100 μM). Both BDE-47 and BDE-49 significantly decreased axonal outgrowth, but these effects were reversed by co-treatment with T3 or NAC. Furthermore NH3, a thyroid hormone receptor antagonist, blocked T3 rescue of BDE-47 inhibition of axon outgrowth. These results suggest that PBDEs can selectively disrupt axonal outgrowth via TH signaling and oxidative stress. Supported by NIH (grants ES014901, P42 ES04699, T32 ES007059) and the US EPA (grant RD835550).

1198 HBCD and PBDEs: Comparisons of the Potential Neurotoxicological Mode of Action of Two Brominated Flame Retardants

L. V. Dishaw, A. Kraft, J. Li, K. Newhouse, A. Luke and S. Rieth. *Office of Research and Development, US EPA, Washington, DC.*

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are brominated flame retardants (BFRs) that have been widely applied to consumer products to meet flammability standards. These chemicals have been found to be persistent, bioaccumulative, and are detected in human breast milk and indoor air and dust. Importantly, current models indicate that young children are likely to have the highest exposure to these and other flame retardants, increasing concern for potential adverse effects during development. Numerous studies in both humans and animal models have reported links between early life exposure to PBDEs and persistent neurobehavioral deficits, including altered performance on cognitive tasks and deficits or delays in motor skills development. Relative to PBDEs, the evidence for neurodevelopmental effects of HBCD is sparse. Some rodent studies suggest that perinatal exposure to this chemical elicits behavioral effects similar to those of PBDEs; however, interpretation of these results is limited by the small size of the database and inconsistencies between studies. A larger database of mechanistic studies indicates that, like PBDEs, potential nervous system effects of HBCD may be mediated by perturbations in calcium and thyroid hormone homeostasis. During early brain development, both calcium and thyroid hormone signals regulate several critical neurodevelopmental processes, including cell proliferation and differentiation, synaptogenesis, neurotransmitter release, and apoptosis. This analysis presents a comparison of the data available for PBDEs and HBCD on these potentially important mechanistic events in light of the developmental neurobehavioral data reported to date, which could help to inform existing data gaps and guide future research into the potential neurodevelopmental effects of HBCD. The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

1199 Diacetyl Causes Age-Enhanced Ubiquitin Aggregation in the Neuroepithelium of the Rat Nose

A. F. Hubbs, A. P. Cole, K. L. Fluharty, L. A. Battelli, P. Charles, M. L. Kashon, A. M. Cumpston, W. T. Goldsmith, M. C. Jackson, W. McKinney, D. G. Frazer, K. Sriram and J. S. Fedan. *National Institute for Occupational Safety and Health, Morgantown, WV.*

Diacetyl is a reactive α-dicarbonyl compound which imparts the aroma and flavor of butter to foods. *In vivo*, diacetyl inhalation causes airway epithelial necrosis in exposed rodents and is implicated in occupational lung disease, particularly obstructive lung disease. In the nose of exposed rodents, diacetyl damages the respiratory epithelium and also the olfactory neuroepithelium, which contains neurons extending into the brain. In addition, diacetyl damages proteins. Damaged proteins are cytotoxic, particularly in neurons, and protein damage often increases with age. To examine the hypothesis that diacetyl damages proteins in the neuroepithelium of the nose in a dose and age-dependent manner, Sprague-Dawley rats inhaled diacetyl at target concentrations of 0, 60, 120, 240 or 320 ppm for 6 h and were sacrificed the following day. The rats were three different ages when exposed: 4-5, 6-7, and 8-9 weeks old. Using indirect immunofluorescence, the neuroepithelium of nose section T2 was stained for ubiquitin aggregation, a hallmark of protein damage. Ubiquitin aggregates were visible as intensely positive immunofluorescent foci within the olfactory neuroepithelium of the nose from diacetyl-exposed rats. The area of ubiquitin/mm basement membrane significantly increased with diacetyl exposure and was dose responsive. Diacetyl-induced ubiquitin accumulation in the neuroepithelium was significantly enhanced by increasing rat age in the 60, 240, and 320 ppm exposure groups. These findings indicate that diacetyl induced olfactory toxicity with protein damage which is enhanced with age.

1200 High Fructose/High Fat Diets Mediate Changes in Protein Carbonyl Content in the Rat Brain with and without Ozone Exposure

J. M. Valdez, J. E. Richards, A. F. Johnstone, J. E. Royland and P. R. S. Kodavanti. *Environmental Public Health, US EPA, Research Triangle Park, NC; Integrated Systems Toxicology, US EPA, Research Triangle Park, NC; Neurotoxicology Branch, US EPA, Research Triangle Park, NC.*

The consumption of diets rich in fat or fructose have been correlated to a rise in type-2 diabetes and obesity. These diet-induced physiological changes have been shown previously to cause an increase in responsiveness to air pollutants such as ozone (O3). O3 is a pervasive air pollutant that has been shown to not only have negative effects on the pulmonary, cardiac, and metabolic systems, but also on the central nervous system. The objective of this study was to measure O3-induced oxidative damage in brain regions of rats fed either normal, high fat, or high fructose diets. Thirty day old male Brown Norway rats were fed diets for 12 weeks prior to and during O3 exposure (5 hr/day, 1 day/wk, for 4 weeks). Frontal cortex (FC), cerebellum (CB), hippocampus (HIP), and hypothalamus (HYP) were dissected, quick frozen on dry ice, and stored at -80 oC. Protein carbonyls (PC) were assayed using commercial kits. Hydrogen peroxide and ferrous ammonium sulfate used as positive controls, increased PC in cortical tissue *in vitro* in a concentration dependent manner. There was an increase in PC due to O3 exposure in the FC of rats on the normal diet, however, this was not observed in the fructose- and fat-fed groups. In contrast, the HIP and HYP of rats on the normal diet showed a decrease in PC following O3 exposure. No other changes were noted between treatments in the HIP, but in the HYP, there was an increase in PC in the high fat diet group. Rats fed the high fat diet showed increased PC production within the CB regardless of treatment. The high fructose diet also increased PC in the CB relative to control diet following O3 exposure. These data show that dietary condition can alter the production of PC in the brain, with region specific effects following O3 exposure. (This abstract does not necessarily reflect US EPA policy).

1201 Effects of Altitude and Toluene on Neuroelectrophysiology in Sprague Dawley Rats

D. A. Mahle[4], S. M. McInturf[2], M. Miklasevich[1], A. Barnett[3] and J. G. Rohan[3]. *[1]CAMRIS, Wright Patterson AFB, OH; [2]NAMRU-D, Wright Patterson AFB, OH; [3]ORISE, Wright Patterson AFB, OH and [4]US Air Force, Wright Patterson AFB, OH.*

Pilots of high performance jets endure unique stresses, including high altitude and exposure to low level volatile organic compounds (VOCs) that have the potential to affect a pilot's air quality. It is unknown what effects exposure to altitude and a VOC would have on cellular functions. Additionally, a number of F-22 pilots were reported to have short-term degraded cognitive function. Adult male Sprague Dawley rats were exposed to air or 200 ppm toluene for 1 hr using a nose only exposure system at simulated altitudes of 940 or 20,000 ft. Oxygen levels at 20,000 ft were maintained at approximately 45% to maintain normoxic piO2. After exposure, brains were rapidly removed and placed in ice cold oxygenated artificial cerebrospinal fluid and then precision sliced to isolate the hippocampus and prefrontal cortex. Electrophysiological measurements were made using a microelectrode array system (MED64), evaluating synaptic transmission, spontaneous activity and synaptic plasticity through long-term potentiation (LTP) and paired pulse stimulation. *In vitro* exposures revealed large transient effects of toluene on paired pulse facilitation of excitatory post-synaptic potentials (EPSP), as well as EPSP amplitudes of hippocampal neurons. However, *in vivo* exposures showed no significant difference in the EPSP characteristics, LTP or spontaneous activity. In the group exposed to high altitude and toluene a difference in amplitude and facilitation of inhibitory post-synaptic potentials (IPSP) of hippocampal neurons was observed, suggesting that the inhibitory pathways of the central nervous system may be more sensitive to toluene effects at high altitude. The payoff is the improved understanding of adverse neurological effects linked to exposure to VOCs under extreme altitude conditions associated with the operation of high performance aircraft.

1202 Histopathological Analysis of the Brain from Mice Treated with Abraxane

T. Maejima, C. Kuwata, T. Ohsawa, I. Igarashi, S. Hakamata, K. Kai and W. Takasaki. *Medicinal Safety Research Laboratories, Daiichisankyo Co., Ltd., Tokyo, Japan.*

Taxane derivatives are widely used as antineoplastic agents. A peripheral sensory neuropathy is the most commonly reported toxicity. However, there are few reports for taxane-induced neurotoxicity in the central nervous system (CNS). In this study histopathological analysis was performed in the brains of mice treated with abraxane (ABX), nanoparticle paclitaxel suspended in human serum albumin. Twelve week-old female BALB/c nude mice (n=5), with human breast cancer xenografts, were intravenously administered ABX at a dose of 150 mg/kg/day every 4 days for 2 cycles (q4dx2). Ten days after the final dosing, animals were euthanized and the brains were collected for histopathological examination. During the dosing period 1 animal died due to ABX-induced toxicity. The four surviving animals had symptoms of peripheral neuropathy such as loss of hindlimb extension reflex. All 4 animals examined histopathologically showed neural necrosis in the brain. The lesions were localized in the hippocampus CA3 and amygdaloid body near lateral ventricle, the region around the anterior horn of the lateral ventricle, the cerebellum and cochlear nucleus near the fourth ventricle, and the region around the third ventricle. These regions, except for the hippocampus CA3, are known as sites where neural progenitor cells or neural stem cells exist. These results indicate that taxane, at least ABX, causes neurotoxicity not only in the peripheral nerves but also in CNS with characteristic distribution of the lesions localized near ventricles.

1203 Assessment of the Neurotoxicity of n-Butanol

D. Segal. *US EPA, Washington, DC.* Sponsor: S. Makris.

n-Butanol is a short-chain alcohol that is structurally similar to ethanol. It is used in many industrial processes, including as an intermediate in polymer production and as a direct solvent. n-Butanol is also a potential biofuel and oxygenate in gasoline. Herein, the potential neurotoxicity of n-butanol is evaluated. Because n-butyl acetate is rapidly converted to n-butanol, n-butyl acetate studies were also considered in this assessment. A comprehensive literature search was conducted for studies evaluating the toxicity of n-butanol and n-butyl acetate through June 2015. The evidence for neurotoxicity and developmental neurotoxicity were considered separately. There is one occupational study that evaluated neurological symptoms in n-butanol exposed workers via inhalation, as well as one subchronic oral exposure study and two subchronic inhalation studies evaluating neurological effects in rodents. Hearing loss was noted in the occupationally exposed workers, and findings of impaired motor coordination and decreased absolute brain weight were seen in exposed rodents (though not consistently observed). Overall, the studies suggest an association between n-butanol exposure and neurotoxicity. Two oral exposure studies and three inhalation exposure studies evaluated developmental neurotoxicity in rats exposed gestationally to n-butanol or n-butyl acetate. These studies suggest that developmental neurotoxicity is also a potential hazard of n-butanol exposure. A study in Wistar rats found dilated brain ventricles in offspring of exposed dams; however, a study in Sprague-Dawley rats did not observe this effect. The three inhalation studies, for the most part, did not find developmental neurotoxic effects in gestationally exposed pups. (Disclaimer: The views expressed in this paper are those of the author and do not necessarily represent the views or policies of the US EPA.)

1204 A Preliminary Analysis of Central Auditory Dysfunction Induced by Jet Fuel Exposures

E. A. Merrill[2], P. J. Robinson[2], T. R. Sterner[2] and D. R. Mattie[1]. *[1]711 HPW/RHDJ, AFRL, WPAFB, OH and [2]AFRL/RHDJ, HJF, WPAFB, OH.* Sponsor: D. Mattie.

Preliminary epidemiological analyses of auditory records from aircraft maintenance personnel exposed to both noise and jet fuel (JP-8) suggest that concurrent exposure may potentiate hearing loss at jet fuel concentrations below the threshold limit value. Recent *in vivo* studies on Long-Evans rats have shown that while exposure to JP-8 (1000 mg/m3) did not induced peripheral hearing loss (i.e. damage to cochlear hair cells), the exposure did impair brainstem encoding of sound intensity 4 weeks after the exposure. These effects were exacerbated when coupled with noise [8 kHz octave band at 85 dB sound pressure level (SPL)]. In addition, several solvents such as toluene and xylene, which are also JP-8 components, have been shown to induce both CNS toxicity and mid-frequency auditory impairments. Mid-frequency hearing impairments have the potential to be more disruptive than high frequency impairments produced by traditional ototoxic compounds because these

frequencies lie closer to those primarily represented in human speech. Little is known regarding the mechanisms of jet fuel induced ototoxicity. Our *in vitro* studies with HEI-OC1 cells (immortalized mouse cochlear epithelial cells) indicate marginal cytotoxicity. Fifty percent lethality was seen at 500 ppm JP-8; however, approximately 40% of the cells remained viable at concentrations as high as 3000 ppm. Proteomic studies at 500 ppm JP-8 revealed upregulation of cell histones involved in chromatin remodeling. Given the minimal effects of JP-8 on peripheral auditory systems, both *in vivo* and *in vitro*, we hypothesize that central auditory processing dysfunctions may be an early manifestation of JP-8 induced ototoxicity. Based on these data and a review of current literature, we have built a preliminary cellular network model that focuses on disruption of the excitatory and inhibitory synaptic connections along the auditory pathway.

1205 Electrophysiological Characterization of Neuronal Activity in *Ex Vivo* Hippocampal Slices Following Repeated *In Vivo* Exposures to Jet Fuel in Rats

J. G. Rohan[2,3], M. K. Miklasevich[1,2], S. M. McInturf[2], C. P. Gut[1,2], M. D. Grimm[1,2], J. E. Reboulet[1,2] and K. L. Mumy[2]. [1]*CAMRIS International, Bethesda, MD;* [2]*Naval Medical Research Unit Dayton, WPAFB, OH and* [3]*Oak Ridge Institute for Science and Education, Oak Ridge, TN.*

Exposure to fuel continues to be a concern in both military and general populations. We combined electrophysiology techniques with behavioral approaches to assess and correlate neurological effects with repeated jet fuel exposure in a rat model. We exposed male rats to 0, 200, 1000 or 2000 mg/m3 concentrations of JP-8 or Jet A jet fuel via whole-body inhalation for 28 days (6 h/d, 5 d/wk). Electrophysiology recording of hippocampal slices was performed within 24 h of the last exposure. We characterized effects of exposure on evoked field responses, synaptic plasticity and spontaneous spiking activity. Behavioral assays were also performed to assess cognitive effects. Electrophysiology findings showed no dramatic differences in hippocampal function after repeated exposure to either JP-8 or Jet A. There was a small but significant difference in evoked field inhibitory response amplitudes surrounding the CA1 and dentate regions between control and exposed groups. There was also a small but significant decrease in paired pulse facilitation from hippocampal neurons within the dentate region of rats exposed to 2000 mg/m3 of Jet A. No significant changes in synaptic plasticity or spontaneous spiking activity were found. Consistent with this, there was no significant deviation in learning and memory or sensorimotor gating as evaluated by the Morris water maze and acoustic startle reflex with pre-pulse inhibition tests, respectively. These data suggest that current occupational exposure limits (200 mg/m3) are not deleterious to the neurotoxicity endpoints evaluated. The subtle differences observed in the rat exposed to high concentrations of jet fuel warrant further investigation into potential or perhaps delayed effects in humans that may not be reflected in the rat model.

1206 Acute Diesel Exhaust Exposure Induces Microglial Activation and Suppresses Adult Hippocampal Neurogenesis but Does Not Induce Apoptosis

J. L. Coburn[2], T. B. Cole[2] and L. G. Costa[2,3]. [1]*Center on Human Development and Disabilities, University of Washington, Seattle, WA;* [2]*Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and* [3]*Department of Neuroscience, University of Parma, Parma, Italy.*

Exposure to environmental pollutants may result in microglial activation, which causes alterations in microglial morphology and increased expression of pro-inflammatory cytokines and chemokines. Signaling between microglia and neurons is important in the proper regulation of adult neurogenesis, and changes associated with microglial activation may disrupt the process. We investigated the effect of diesel exhaust (DE) on microglia activation and neurogenesis in the hippocampus of mice. Male and female adult mice were treated with the proliferating cell marker bromodeoxyuridine (BrdU), and were then exposed to filtered air (FA) or DE at a concentration of approximately 250 µg/m3 for 6 hours. Fixed, frozen brains were sectioned and stained for the microglial marker Iba1 (to measure microglia activation), cleaved caspase-3 (to measure apoptotic cell death), or the neuronal nuclear marker NeuN and BrdU (to assess adult neurogenesis in the subgranular zone). Microglial activation was assessed by comparing soma area and circularity between treatment groups and genders. Area of microglial soma was increased in DE-exposed animals (females, FA 41.4±0.5µm2 DE 52.2±1.3µm2; males, FA 40.0±0.6µm2 DE 56.8±1.1µm2 ***p<0.001), while circularity was reduced (females, FA 0.66±0.01, DE 0.58±0.02; males FA 0.67±0.02 DE 0.59±0.01 *p<0.05). Hippocampal neurogenesis was significantly reduced in both genders, with a larger reduction observed in males (FA 0.68±0.03, DE 0.25±0.06, ***p<0.001) than females (FA 0.43±0.04, DE 0.26±0.03, *p<0.05). Apoptosis in the hippocampus did not differ between FA and DE in either males or females. These findings suggest that acute DE exposure, by inducing microglial activation, may suppress neurogenesis by reducing proliferation without affecting cell survival. (Supp. in part by R01ES022949)

1207 Microglia Modulate the Effect of Diesel Exhaust Particles on Neuronal Death: Role of Pro-inflammatory Cytokines

P. J. Roque[2] and L. G. Costa[1,2]. [1]*University of Parma, Parma, Italy and* [2]*Dept of Environmental & Occupational Health Sciences, University of Washington, Seattle, WA.*

In addition to the well-established effects of air pollution on the cardiovascular and respiratory systems, emerging evidence has implicated it in inducing negative effects on the central nervous system. Diesel exhaust particulate matter (DEP), a major component of air pollution, is a complex mixture of numerous toxicants. Limited studies have shown that DEP-induced dopaminergic neuron dysfunction is mediated by microglia, the resident immune cells of the brain. We show that mouse microglia play a similar role in mediating primary cerebellar granule neuron (CGN) death. While DEP (0, 25, 50, 100 µg/2cm2) has no effect on CGN viability after 24 hours of treatment, in the presence of primary cortical microglia neuronal cell death significantly increased by a factor of 2 to 3 after co-treatment with DEP (50, 100 µg/2cm2), suggesting that microglia are important contributors to DEP-induced CGN neurotoxicity. Activated microglia release reactive oxygen species and pro-inflammatory mediators. DEP treatment (50 µg/2cm2) of primary microglia for 24 hours results in morphological changes indicative of microglia activation. Further, blocking microglia activation in the neuron-microglia co-culture system with minocycline (50 µM) attenuated DEP-induced CGN death. We hypothesized that microglia-mediated neuroinflammation may play a role in the DEP effect on CGN viability. The peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist pioglitazone has been shown to have anti-inflammatory effects on microglia. Co-treatment of microglia and CGNs with DEP (50 µg/2cm2) in the presence of pioglitazone (50 µM) attenuated neuronal cell death. Additionally, mRNA levels of the pro-inflammatory cytokines IL-6 and IL1-β, as measured by qPCR were increased in microglia after 24 hours of DEP treatment (50 µg/2cm2) and IL-6 release, as measured by ELISA, also increased. Together, these data show that microglia-induced neuroinflammation may play a critical role in modulating the effect of DEP on neuronal viability. (Supp. in part by R01ES22949).

1208 Functional Neurotoxicity Evaluation of Noribogaine Using Video-EEG in Cynomolgus Monkeys

S. Authier[2], M. Pouliot[2], D. Paquette[1], R. Kubaszky[2], J. Arezzo[7], R. J. Stubbs[6], R. J. Gerson[4], L. T. Friedhofff[5] and H. Weis[3]. [1]*Centre Vétérinaire DMV, Montréal, QC, Canada;* [2]*CiToxLAB North America, Laval, QC, Canada;* [3]*DemeRx, Inc., Fort Lauderdale, FL;* [4]*Gerson Pharma Solutions, LLC, Lincoln University, PA;* [5]*Lawrence T Friedhofff, River Vale, NJ;* [6]*Stubbs & Hensel Pharma Consulting, LLC, Blue Bell, PA and* [7]*Yeshiva University, Albert Einstein College of Medicine, New York, NY.*

Introduction: Continuous Video-EEG monitoring remains the gold standard for seizure liability assessments. Noribogaine is an iboga alkaloid currently in clinical trials for the treatment of opioid dependence. Methods: EEG traces were obtained in cynomolgus monkeys (3 males and 3 females) using implanted electrodes, telemetry and bipolar derivations from the 10-20 system (C3-O1, C4-O2 and Cz-Oz). Vehicle and noribogaine (160 and 320 mg/kg) were administered orally in a dose escalation design with blood samples obtained using a remote collection system. Pentylenetetrazol (PTZ)(IV, 40 mg/kg/hr) was used as a positive control as the last treatment. Results: At doses of 160 mg/kg, Noribogaine was associated with a variety of clinical signs including chewing, emesis, scratching, licking, partial anorexia, slightly decreased activity level, ataxia, myoclonic jerks and/or whole body tremors. These behaviors were not associated with any EEG abnormality. Increasing the dose of Noribogaine to 320 mg/kg raised the incidence of the outlined behaviors; however, the concomitant EEG remained "within normal limits" for all monkeys. A single brief episode (<3 sec) of paroxysmal activity was observed in one monkey given 320 mg/kg but this was correlated with EMG artifacts. Mean plasma concentrations (Cmax) obtained at 320 mg/kg dose were 1,133 and 1,003 ng/mL in males and females, respectively, with a terminal elimination half-life of 4.4 hr. These plasma

levels are substantially higher than the levels intended to be reached in ongoing clinical studies. PTZ was invariably associated with EEG paroxysmal activity at an average dose of 48 mg/kg and EEG seizure activity at an average dose of 54 mg/kg. Conclusion: There was no evidence of drug-related EEG changes following administration of Noribogaine at doses up to 320 mg/kg. However, there were concurrent clinical signs that appeared to be related to central nervous system effects, which correlated with plasma exposures and resolved by the end of the monitoring period.

PS 1209 Redox Control of Neuroinflammation by Post-Translational Activation of Glutamate Cysteine Ligase

P. B. McElroy[2], L.-P. Liang[2], B. J. Day[1] and M. Patel[2]. [1]*Department of Medicine, National Jewish Health, Denver, CO and* [2]*Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO.*

Neuroinflammation and oxidative stress are hallmarks of neurological diseases linked to toxicant exposure. However, whether and how the redox processes control neuroinflammation is relatively unknown. We hypothesized that elevating cellular glutathione (GSH) levels would inhibit neuroinflammation. Cellular GSH levels were elevated by a novel approach i.e. post-translational activation of glutamate cysteine ligase (GCL), the rate-limiting enzyme in GSH biosynthesis. A series of thiol-containing compounds were examined for their ability to increase intracellular GSH levels in a murine microglial cell line (BV2), of which 2,3-dimercapto-1-propanol (DMP) was found to be the most potent compound. DMP increased GCL activity and decreased LPS-induced production of an array of pro-inflammatory cytokines and iNOS induction in BV2 cells in a concentration-dependent manner. Additionally, DMP's ability to elevate GSH levels and attenuate LPS induced pro-inflammatory cytokine production was inhibited by buthionine sulfoximide, an inhibitor of GCL activity and GSH biosynthesis. DMP also increased the expression of GCL holoenzyme without affecting the expression of the subunits GCLC and GCLM or that of other Nrf2 target proteins (NQO1 and HO-1), suggesting a post-translational mechanism. Moreover, DMP attenuated LPS-induced MAP kinase activation in BV2 cells. Finally, we determined if DMP increased GSH levels and attenuated neuronal damage in a rat dopaminergic N27 cell line. DMP treatment in N27 cells increased GCL activity and GSH levels similar to the magnitude observed in BV2 cells. Furthermore, DMP inhibited cell death induced in N27 cells by paraquat, a model environmental toxicant. Together, the data demonstrate that elevation of intracellular GSH levels by post-translational activation of GCL inhibits production of pro-inflammatory cytokines and exerts neuroprotection. It also suggests that post-translational activation of GCL is a novel approach to target inflammation and cell death in neuronal disorders where adaptive responses may be impaired.
Grant support: RO1 NS045748 (M.P)

PS 1210 Ameliorative Effect of Propolis and Ginger versus Neurotoxicity and Oxidative Stress Evoked by Monosodium Glutamate in Male Albino Rats

N. E.-H. Y. Awad[2], M. E. Elhalwagy[2] and A. R. Zaki[2]. [1]*Department of Mammalian Toxicology , Pesticides Central Laboratory , Agriculture Research Center,Giz, Beni-sueif University- Cairo, Egypt, Egypt and* [2]*Forensic Medicine and Toxicology, Beni-sueif University- Cairo, Egypt, Egypt.*

Monosodium glutamate (MSG) is a popular flavour used in food industries; excess MSG is neurotoxic. Oxidative stress is well documented in MSG induced neurotoxicity. This present study has been designed to evaluate the neuroprotective effects of both Proplis and Ginger compounds on MSG-induced neurotoxicity in rats. Fourty adult male albino rats were divided into 4 groups (each containing 10 rats). Group I (control); were orally administrated with normal saline. Group II (MSG), were orally administrated with MSG (830 mg/ Kg. B. wt). Group III (MSG + Proplis); were orally administrated with MSG and subsequently by Proplis (500 mg/kg B. wt). Group IV (MSG+ Ginger); were orally administrated with MSG and subsequently by Ginger (600 mg/kg). At the end of the treatment period (60 days in all groups), blood samples and brain tissue were collected for estimation of LPO and measurement of antioxidant status of glutathione, catalase and superoxide dismutase. Estimation of calcium, sodium and potassium ions in brain tissue and gamma aminobutyric acid level in serum was carried out. The histopathological study of brain tissue was also carried out. MSG caused a significant alteration in oxidative defense; raised levels of LPO and depletion of antioxidant levels. There were neurogenerative changes in the form of vacuolization, pyknosis and congestion in the cerebral cortex. Moreover MSG treatment induced up regulation of Bax protein in brain tissue indicating that MSG induced apoptosis. Treatment with both Propolis and Ginger significantly attenuated oxidative stress, and cerebral damage in MSG-treated animals, also significantly reduced the monosodium glutamate-induced excitotoxicity by decreasing the level of Ca (+2) and Na(+) with concomitant increase in the level of K(+). Serum gamma aminobutyric acid level was also increased in Proplis and Ginger treated animals. The histopathological evidence supports the neuroprotective activity of both. Hence, this study demonstrates that propolis and ginger possess beneficial effects against various neurotoxic insults induced by MSG in rats, returned to their antioxidant and anti-inflammatory properties.

PS 1211 Microglia Are Biosensors of Neuroinflammogens and Neurotoxicity, Whereas Astrocytes Are Linked Only to Neurotoxicity

J. P. O'Callaghan, K. A. Kelly, A. R. Locker, L. T. Michalovicz and D. B. Miller. *CDC/NIOSH, Morgantown, WV.*

A characteristic feature of neurotoxicity is the selective and unpredictable damage to specific neural cells. This lack of target identity constitutes a major barrier to neurotoxicity detection. Evaluating astrogliosis and microglial activation overcomes this problem as these glial cell types react to neurotoxicant exposures to reveal sites of CNS damage. Thus, astroglial and microglial biomarkers often are used as indices of neurotoxicity. Previously we showed that damage from diverse neurotoxicants initiates microglial associated neuroinflammation, the subsequent activation of STAT3 and the induction of GFAP. These findings are indicative of a link between microglial-related neuroinflammation and astrogliosis. Nevertheless, anti-inflammatory treatment with minocycline can suppress neuroinflammation instigated by neurotoxicity without suppressing astrogliosis. Given these observations, it seemed possible that indices of neuroinflammation could be dissociated from neural damage and astrogliosis, despite the fact that multiple neurotoxicity models result in STAT3 activation temporally linked to induction of GFAP. To test this possibility, we employed an acute exposure to the known inflammogen, LPS, to induce neuroinflammation. Our prior data revealed that systemic administration of LPS (2mg/kg, s.c.) did not cause neurotoxicity or astrogliosis in any brain region but did result in brain-wide expression of microglia-associated proinflammatory cytokines/chemokines, Tnf-α, Osm, Ccl2, and Lif, as well as TSPO, a known micoroglial marker of neurotoxicity. LPS also activated STAT3 over a 12-hr post exposure period, when proinflammatory cytokine levels resolved to near baseline. Activation of STAT3 by LPS, unlike the activation associated with multiple models of neurotoxicity, was suppressible by acute pretreatment with the anti-inflammatory glucocorticoid, corticosterone. Neuroinflammation, expression of TSPO, and activation of STAT3 resulting from LPS did not affect the expression of GFAP in any brain region over a 72-hour time period. Together, these data serve to indicate that "acute phase" neuroinflammation caused by LPS can induce TSPO and activate STAT3 without resulting in neural damage or astrogliosis. The STAT3 pathway appears to serve as a dual "switch" for mediating acute neuroinflammatory responses separate from its role in mediating damage-induced astrogliosis.

PS 1212 Corticosterone Priming of the Neuroinflammatory Response to AChE Inhibitors Results in Overexpression of Tlr2 and Downstream Targets, but Not Activation of the Nlrp3 Inflammasome

L. T. Michalovicz, K. A. Kelly, A. R. Locker, D. B. Miller and J. P. O'Callaghan. *CDC/NIOSH, Morgantown, WV.*

Previously, we have shown that exposure to acetylcholinesterase inhibitors (AChEIs) produces inflammation in the mouse brain. Furthermore, this inflammation is greatly exacerbated, or "primed", by prior, chronic exposure to corticosterone (CORT). Inflammatory priming is a phenomenon commonly associated with the inflammasome, a component of the innate immune system comprised of a multitude of proteins including pattern recognition receptors, cytokines, caspases and other adaptor proteins. Prior work evaluating the response to chronic CORT exposure in rats found evidence for activation of the NLRP3 inflammasome. In this experiment, we investigated the involvement of the NLRP3 inflammasome in the neuroinflammatory response to the irreversible AChEIs, diisopropyl fluorophosphate (DFP) and chlorpyrifos (CPF), and the reversible AChEI, pyridostigmine bromide (PB), in male C57BL/6 mice with and without chronic (4 days) exposure to CORT (200 mg/L) in the drinking water prior to AChEI treatment. Here, we found the gene expression of toll-like receptor 2 (TLR2) and its downstream transcriptional targets, S100A8 and S100A9, to be increased in response to CORT DFP and CORT CPO 6 hrs after exposure, while TLR4 and NLRP3 expression were unaf-

fected. However, NLRP3 expression was increased when CORT exposure was extended to 7 days of treatment. Past studies in our lab revealed that PB alone or with CORT pretreatment do not produce neuroinflammation, thus it is not surprising that we found no effect on any of these genes of interest following exposure to PB. Interestingly, expression of serum amyloid a (SAA1), an endogenous ligand of TLR2, was not increased by DFP or CPF, with or without prior CORT exposure. Our data indicate potential involvement of TLR2 signaling in the CORT-priming of the neuroinflammatory response to the AChEIs, DFP and CPF. However, the enhanced expression of inflammatory cytokines in the brain following exposure to CORT and these AChEIs is independent of NLRP3 expression, which appears to be a primary effect of prolonged CORT exposure. By further examining the role of TLR2 in an augmented neuroinflammatory response following the combined CORT and AChEI exposure, we can identify potential targets for the treatment of illness associated with these exposures, such as Gulf War Illness.

PS 1213 Revealing Behavioral Learning Deficit Phenotypes Subsequent to *In Utero* Exposure to Benzo(a)pyrene

M. M. McCallister[3], Z. Li[3], T. Zhang[5], A. Ramesh[3], R. S. Clark[3], M. Maguire[3], B. Hutsell[4], C. M. Newland[4] and D. B. Hood[2,5]. *[1]Department of Biochemistry and Cancer Biology, Meharry Medical College, Nashville, TN; [2]Department of Neuroscience, College of Medicine, The Ohio State University, Columbus, OH; [3]Department of Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN; [4]Department of Psychology, Auburn University, Auburn, AL and [5]Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, OH.*

To characterize behavioral deficits in pre-adolescent offspring exposed *in utero* to B(a)P, timed-pregnant Long Evans Hooded rats were treated daily with B(a)P (150, 300, 600 and 1200µg/kg BW) or peanut oil (vehicle) on E14,15,16, and 17. Following birth, but during the pre-weaning period, B(a)P metabolites were examined in plasma and whole brain or cerebral cortex from exposed and control offspring. Tissue concentrations of B(a)P metabolites were 1) dose-dependent and 2) followed a time-dependence for elimination with an approximate 60% reduction by PND5 in the 1200µg/kg BW experimental group. Spatial discrimination-reversal learning was utilized to evaluate potential behavioral neurotoxicity in P40-P60 offspring. Late-adolescent offspring exposed *in utero* to 600 and 1200 µg/kg BW were indistinguishable from their control counterparts in the ability to acquire an original discrimination and reach criterion. However, a dose-dependent effect of *in utero* B(a)P-exposure was evident upon a discrimination reversal as exposed offspring perseverated on the previously correct response. This newly characterized behavioral deficit phenotype for the 1st reversal was not apparent in either the 1) original discrimination or 2) subsequent reversal sessions relative to the respective control offspring. This is the first such documented report of a spatial discrimination reversal deficit after *in utero* exposure to B(a)P. Furthermore, the expression of activity related-cytoskeletal associated protein (Arc), an experience-dependent cortical protein marker known to be up-regulated in response to acquisition of a novel behavior, was greater in B(a)P exposed offspring included in the spatial discrimination cohort versus home cage controls. Collectively, these findings support the hypothesis that *in utero* exposure to B(a)P during critical windows of development representing peak periods of neurogenesis results in behavioral deficits in later life.

PS 1214 Exploration of the Gulf War Illness Phenotype in a Mouse Model Challenged with LPS at Long Term Time Points

K. A. Kelly, A. R. Locker, L. T. Michalovicz, D. B. Miller and J. P. O'Callaghan. *CDC/NIOSH, Morgantown, WV.*

Chronic exposure to the glucocorticoid, corticosterone (CORT), at high physiological stress levels, can prime the CNS to release proinflammatory cytokines following exposure to neurotoxic exposures and systemic inflammation. Such neuroinflammatory events are associated with sickness behavior. Gulf War Illness (GWI) is a multi-symptom disorder characterized by persistent headaches, chronic fatigue, muscle pain, memory loss, confusion, gastrointestinal problems, and rashes, features also characteristic of persistent sickness behavior. Recently we found that, of the multiple exposures soldiers faced in the 1991 Gulf War theatre, two produced exacerbated neuroinflammation in mice. By mimicking the stresses of war with high physiologic exposure to CORT (200 mg/L 0.6% EtOH drinking water) over 7 days followed by sarin gas exposure with surrogate acetylcholinesterase inhibitor, diisopropyl fluorophosphate (DFP; 4 mg/kg, i.p.), we found heightened neuroinflammatory responses without evidence of astrogliosis or neurodegeneration 6 to 72 hours after DFP exposure. While these observations recapitulated the early symptoms of GWI, the essential pathobiology of GWI is the persistence of heightened responses to external stimuli for the past 20+ years. Here we employed episodic exposure of CORT in the drinking water of C57Bl6/J male mice for up to 180 days (CORT drinking water for 4 or 7 days every other week) to emulate episodic stress incurred by ill veterans following their exposures to multiple agents in theater, including sarin, 2 decades ago. Systemic exposure to LPS, at sub- and neuroinflammatory doses, were used to challenge the GWI phenotype (0.5 or 2 mg/kg, s.c., respectively). Our results show that successive waves of CORT provide a priming of the neuroinflammatory response to LPS, and that DFP (single exposure on the last day of the first CORT treatment) can exacerbate this effect. Thus, confirming this mouse model to produce a similar phenotype as that seen in soldiers diagnosed with GWI. Interestingly, we found that while 4 days of CORT in the drinking water is sufficient to produce an exacerbated neuroinflammatory response to LPS, that successive waves of CORT in 7 day, but not 4 day, exposures, every other week for 90 days produces a synergistic priming of the CNS to greatly augment the neuroinflammatory response to LPS. Together, these data suggest that GWI is a chronic, stressor primed, neuroinflammatory condition.

PS 1215 Comparison of Nerve Excitability, Nerve Conduction Velocity, and Behavioral Tests for Acrylamide-Induced Peripheral Neuropathy

D. L. Freeborn, G. L. Jung, K. L. McDaniel, V. C. Moser and D. W. Herr. *Neurotoxicology, US EPA, Durham, NC.*

Nerve excitability (NE) testing is a sensitive method to test for peripheral neurotoxicity in humans, and may be more sensitive than compound nerve action potential (CNAP) or nerve conduction velocity (NCV). We used acrylamide to compare the NE and CNAP/NCV methods. Behavioral tests known to be sensitive to acrylamide-induced neurotoxicity were included. Male Long-Evans rats aged 10-11 weeks were given acrylamide in their drinking water at 0, 1.5, 2.5 or 3.0 mM for 6 weeks. These concentrations resulted in an average exposure of 0, 10.3, 14.7, and 14.4 mg/kg/day of acrylamide. NE testing occurred during the 5th week, and CNAP/NCV testing was during the 6th week of treatment. Only 5 animals in the 3.0 mM group were tested due to pronounced neuromuscular and respiratory toxicity, and were not included in statistical analysis. Behavioral testing occurred weekly during treatment, prior to electrophysiology testing, and included open-field evaluations and measures of forelimb/hindlimb grip strength and landing foot splay. Both 1.5 and 2.5 mM treatments altered measures of neuromuscular and sensory function, including increased splay, lower hindlimb grip strength and rearing, and impaired gait and righting reflex. These changes worsened over the 6 weeks of exposure. NE tests indicated decreased nerve excitability, possibly coupled with altered K+ channel activation in sciatic motor nerves and tail mixed nerves. The NE changes were observed at the 2.5 mM treatment concentration. Changes in tail motor nerve NE and CNAPs/NCV in tail mixed nerves were not significant. Results indicate that for acrylamide-induced peripheral neurotoxicity, NE testing detected changes in physiological function, which differed between types of nerves, and was more sensitive than traditional CNAP/NCV methods. Behavioral changes were consistent with previous studies. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

PS 1216 Exposure to Fine Particulate Matter Increases Glutamate Uptake in Bergmann Glia Cells

E. Suárez, A. De Vizcaya-Ruiz and A. Ortega. *Departamento de Toxicología, Cinvestav, Distrito Federal, Mexico.*

Exposure to urban airborne particulate matter (PM) is associated with adverse health effects, and outcomes in the central nervous system have become a relevant public health issue. Recent studies have linked exposure to fine particulate matter to dysfunction of the glutamatergic neurotransmission, given the fact that its components can reach the brain and instigate damage locally or systemically. Glutamate, the main excitatory amino acid transmitter triggers a wide variety of signal transduction cascades that regulate protein synthesis at the transcriptional and translational levels. Activity dependent differential gene expression has been attributed to the activation of both membrane glutamate receptors and transporters. The bulk of glutamate uptake takes place in glia cells. Within the cerebellum, Bergmann glia cells (BGC) are responsible for most of the glutamate uptake activity through the Na+ dependent glutamate/aspartate transporter (GLAST/EAAT-1). Taking into consideration the functional role of Bergmann glia, in terms of the recycling of glutamate, the supply to neurons of lactate, and the prevention of neurotoxic insults, we decided to investigate if air pollution fine particulate matter (PM2.5) exposure affects glia cells that surround glutamater-

gic synapses and by these means alter the major excitatory transmitter system in the brain. To this end, we exposed cultured chick cerebellar Bergmann glia cells to fine PM2.5 isolated and concentrated from Mexico City's northern region (high traffic and industrial activity area) and measured the [3H]-D-Aspartate uptake activity. Our results show a time and dose dependent modulation of uptake activity. Furthermore, PM2.5 treatment resulted in the activation of the mitogen-activated protein kinases (MAPK) independent of p60src (non-receptor tyrosine kinase p60src). These results suggest that glial cells are involved in the deleterious effects in the CNS from exposure to air pollutants, strengthening the notion of the critical role of these cells in brain function.

PS 1217 Exposure to Airborne Ambient Ultrafine Particulate Matter Alters the Expression of Several Noncoding RNAs and Metallothioneins in Primary Human Neurons

P. Solaimani[2], A. Saffari[1], C. Sioutas[1] and A. Campbell[2]. [1]*Department of Civil and Environmental Engineering, University of Southern California, Los Angeles, CA and* [2]*Pharmaceutical Sciences, Western University of Health Sciences, Pomona, CA.*

Exposure to ambient airborne particulate matter has been associated with an increased risk of neurodevelopmental disorders and neurodegenerative diseases, but the mechanism by which this occurs remains unclear. In this investigative study, we sought to gain insight into this potential neurotoxicity by looking at global gene expression changes caused by exposure to ultrafine particulate matter. Microarray analysis was performed on primary human neurons after a 24 hour exposure to an aqueous suspension of 20µg/mL ultrafine particles collected from downtown Los Angeles. Overall, we found a significant change in 128 transcripts. Remarkably, we found an enrichment in our data. The impact of ultrafine particles on 102 of the 128 hits was on the expression of noncoding RNAs. Noncoding RNAs are involved in epigenetic regulation of gene expression and changes in the expression of these noncoding RNAs could impact the function of several other protein coding gene targets. In addition, a significant increase in the expression of metallothionein proteins 1A and 1F was detected after exposure to particulate matter as confirmed by quantitative real-time polymerase chain reaction. Metallothionein 1 proteins are responsible for neuroprotection after exposure to environmental insult. However, prolonged induction of MT1's can have adverse effects. For example, sustained MT1 expression has been associated with plaque formation in Alzheimer's disease patients. The results from these studies reveal that ultrafine particulate matter causes changes in gene expression, which over time may contribute to the pathogenesis of disorders of the central nervous system.

PS 1218 Neurotoxic Effect of the Alkyl Polycyclic Aromatic Hydrocarbons in Human Neuroblastoma Cells

S. N. Sarma, J. M. Blais and H. M. Chan. *Department of Biology, University of Ottawa, Ottawa, ON, Canada.*

Alkylated polycyclic aromatic hydrocarbons (Alkyl-PAH) are the major constitute of total PAH in crude oil and originated from incomplete combustion process. The potential differential toxicity and related mechanism are not known. In this study, we used two abundant Alkyl-PAHs (Dibenzothiophene and Retene) and a well-studied parent PAH (Benzo[a]pyrene) to elucidate the mechanism of neurotoxicity using human neuroblastoma cells. The SK-N-SH neuroblastoma cells were differentiated into neuronal cells and exposed with the different concentration for 24 hours to obtain the cytotoxic concentrations. The 10 % and 20% lethal concentration (LC10 & LC20) were found at around 10 µM and 40 µM. Differentiated neuronal cells were exposed with the PAHs treatments showed significant dose-dependent increase levels of reactive oxygen species, superoxide dismutase activity and the amount of lipid hydroperoxide. Alkyl-PAHs showed to be more toxic than the parent PAH and more potent in inducing all the end point measured. The exposure of alkyl-PAHs with sub-lethal concentration (500 nM) also increased the reactive oxygen species level and superoxide dismutase activity. Our present data showed that the benzo[a]pyrene, dibenzothiophene and retene may induce the neurotoxicity by the redox signaling pathway. The western blot analysis and gene expression profiles will be analyzed to elucidate the pathway of neurotoxicity.

PS 1219 Circulating NGF Increases by Systemic Oxidative Stress and Orchestrates an Antioxidant Response via the TrkA/Akt Pathway in the Central Nervous System

C. E. Garza-Lombo, C. Valdovinos-Flores, J. H. Limon-Pacheco, R. Leon-Rodriguez, P. Petrosyan and M. E. Gonsebatt. *Medicina Genomica y Toxicologia Ambiental, Universidad Nacional Autonoma de Mexico, Mexico, Mexico.*

The central nervous system (CNS) contains a limited amount of glutathione (GSH) and is susceptible to oxidative injury. The systemic inhibition of GSH synthesis by L-buthionine-S-R-sulfoximine (BSO) in mouse, triggers a protective response increasing CNS GSH content. Environmental factors such as diet and life style can induce systemic oxidative stress linked to an increased synthesis of nerve growth factor (NGF). NGF is an important activator of antioxidant mechanisms via the tropomyosin-related kinase receptor A (TrkA). To investigate the mechanisms involved in the increase of brain GSH, we treated Balb/c male mice with BSO or arsenite. Animals were sacrificied 0.5, 2, 6 or 24 h after treatment. We observed the activation of the NGF/TrkA pathway in the mouse striatum via the TrkA/Akt/Nrf2 pathway, upregulating the transcription of gclm gene, implicated in GSH synthesis and nrf2 the transcription factor involved in antioxidant responses. At the same time, the amino acid transport systems responsible for the uptake of L-cys or L-cys2 (xct, lat1 and eaac1) for GSH synthesis were upregulated. Our findings suggest the NGF/TrkA/Akt/Nrf2 pathway as a modulator of a neuroprotective response to systemic oxidative stress in those CNS structures where this NGF receptor is expressed.

PS 1220 Monitoring Calcium Fluxes from Cerebral Cortical Cultures to Identify Effective Treatments Against GABA-A Receptor Channel Poisons

M. Laukova[2], J. Veliskova[2], P. K. Stanton[2], L. Velisek[2] and M. P. Shakarjian[2,3]. [1]*Cell Biology and Anatomy, New York Medical College, Valhalla, NY;* [2]*Environmental Health Science, New York Medical College, Valhalla, NY and* [3]*Medicine, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ.*

The synthetic rodenticide, tetramethylenedisulfotetramine (TMDT) and the naturally-occurring sesquiterpene lactone, picrotoxin (PTX), cause their seizure-producing neurotoxic actions by impeding chloride entry through the GABA-A receptor chloride channel. As a consequence, GABA signaling is impaired, and brain excitatory transmission prevails. This can lead to calcium-dependent excitotoxicity. We sought to monitor changes in intracellular calcium as a marker for GABA-A receptor channel poison action and its alleviation by candidate anti-seizure treatments. Cells from embryonic day 18 rat cerebral cortices were cultured in neurobasal medium for 9 - 14 days and loaded with calcium-sensitive fluorescent dye. Intracellular fluorescence monitoring revealed spontaneous calcium oscillations (SCO). These SCOs were concentration-dependently reduced by GABA-A receptor agonist muscimol. Conversely, TMDT and PTX reduced frequency and increased amplitude of the SCOs. GABA-A receptor positive modulators and NMDA receptor antagonists, both of which relieve TMDT and PTX toxicity *in vivo*, could reverse the actions of the poisons on calcium fluxes *in vitro*, however the former had a more transient, and the latter a more prolonged action. This suggests that NMDA receptor antagonists may offer more prolonged protection from toxicity of the GABA-A receptor channel poisons than other treatments. In conclusion, we describe an *in vitro* method for identifying potential treatments for GABA-A receptor channel poisons and show differential actions of treatments from two different pharmacological classes. Supported by NIH R21NS084900.

PS 1221 Gender Differences in Expression of ATP-Binding Cassette Transporters at Blood-Brain Interfaces in Response to a Hepatotoxic Dose of Acetaminophen

K. M. Flores[2], J. R. Renfro[2] and J. Manautou[2]. [1]*Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT and* [2]*Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT.*

The choroid plexus (CP) or blood-cerebrospinal fluid barrier (BCSFB) and blood-brain barrier (BBB) control the movement of drugs and metabolites between the brain and systemic circulation. Gender differences in drug excretion and detoxification mechanism can affect therapeutic and toxic responses. For example, male mice are more susceptible to acetaminophen (APAP) induced hepatotoxicity. The ATP-binding cassette transporters (Abc transporters) and nuclear factor erythroid-2-related

factor (Nrf2) play a role in the susceptibility to APAP induced hepatotoxicity. Our aim was to examine gender differences in expression of Abc transporters, Nrf2 and related stress genes (NAD(P)H quionine oxidoreductase-1, Nqo1 and heme oxigenase-1, Ho-1) in the brain and CP. We hypothesized that hepatic/renal gender-specific patterns of these cytoprotective genes would also be present in the blood brain interfaces. In female mice, as compared to male, Abcc1-2 and Abcc4 transporters are more strongly expressed brain, supporting our hypothesis. qRT-PCR analysis revealed higher expression of Abcc1-2 and Nrf2 in the female brain, but no differences in the CP. Abcc4, Ho-1 and Nqo1 showed higher expression in female in both brain interfaces. These differences may contribute to APAP potential for neurotoxicity. We examined the differences in mRNA expression of cytoprotective genes at the brain interfaces of WT and Nrf2-/- mice following a hepatotoxic dose of APAP (400mg/kg). APAP enhanced the expression of Abcc2 and Abcc4 in brains of male WT mice, but reduced the expression of these transporters in male Nrf2-/- mice. No changes were observed in the brains of either genotype of female mice. These data suggest that the expression of these transporters in the female mouse brain are not altered by a hepatotoxic dose of APAP and that transcription factors other than Nrf2 may be responsible for the regulation of Abc transporters in the female brain. (Supported by NSF and NIH)

1222 *In Vitro* Assessment of Human Neurotoxicity via High Content Analysis of Neurite Outgrowth Inhibition in SHSY5Y Cells and Induced Pluripotent Stem Cell (iPSc) Derived Neurons

P. Hewitt, A. Kunz, J. Sebbel and C. van Amsterdam. *Non Clinical Safety, Merck KGaA, Darmstadt, Germany*.

Here we present a comparative study for the improvement of the *in vitro* assessment of human neurotoxicity in the early stages of drug development. The SH-SY5Y human neuroblastoma cell line and iPSC derived neurons were chosen as neuronal cell systems. 33 Compounds were selected for validation of the model, including 22 positive substances in which human neurotoxicity *in vivo* was observed. For a robust performance the chosen positive compounds covered a wide range of mechanisms of action and toxicity - including microtubule stabilization/destabilization, proteasome inhibition, protein kinase inhibition and platinum-DNA adduct formation. Seven non-neurotoxic compounds and 4 compounds which influence neuronal function were included. A sequential differentiation protocol using retinoic acid followed by brain-derived neurotropic factor was used for SH-SY5Y cell differentiation. Commercially available iPSC derived neurons (Peri.4U neurons, from Axiogenesis) were differentiated according to the manufacturer's instructions. Cells were treated at multiple concentrations for 72 hours, and subsequently cells were fixed and stained with Hoechst 33342 nuclear stain, rabbit anti βIII-Tubulin antibody and Alexa Fluor® 488 conjugate anti-rabbit antibody. Image acquisition and quantification was carried out using Thermo Arrayscan® VTI HCS Reader in combination with HCS Studio Software. For Peri.4U neurons a parallel assessment of changes in neurite length for neurotoxicity and neuron count for cytotoxicity was applied. In summary, the sensitivity (false negatives) and specificity (false positives) in both cell systems was 59% and 100%, respectively. In Peri.4U neurons these effects occurred at concentrations that were not cytotoxic and generally lower than in SH-SY5Y cells. In conclusion, Peri.4U neurons and SH-SY5Y cells are suitable models for use in early stage preclinical safety assessment of neurotoxicity. However, specific mechanisms of *in vivo* neurotoxicity are not picked up.

1223 Molecular Insights into Pharmacological Inhibition of Microglial Pro-Inflammatory Response Using Mass Spectrometry-Based Proteomics

A. Morris[1], J. Guergues[2], D. Chaput[2] and S. M. Stevens[2]. [1]*Rider University, Lawrenceville, NJ and* [2]*University of South Florida, Tampa, FL*. Sponsor: R. Pollenz.

Microglia, the resident immune cells of the brain, have been shown to be a major source of neuroinflammation as this cell type can become over activated to produce ROS and other neurotoxic factors in the presence of injured neurons. Using an unbiased, global-scale protein expression profiling approach, we investigated the effect of a histone demethylase inhibitor, PBIT, on the microglial pro-inflammatory response induced by the endotoxin, lipopolysaccharide (LPS), in mouse microglial (BV2) cells. Proteomic analysis using iTRAQ allowed for multiplexed relative quantitation of protein expression of over 4,600 proteins in BV2 cells including coverage of proteins relevant to the immune response in microglia. PBIT co-treatment with LPS affected approximately 10% of the measured proteome compared to LPS treatment where several proteins and pathways related to the blunted pro-inflammatory response by PBIT were identified including decreased levels of the immunoresponsive gene IRG1 (also, cis-aconitate decarboxylase). In addition to proteomic analysis, a targeted cytokine assay was performed and confirmed that PBIT reduced the LPS-induced pro-inflammatory response as measured by cytokine release of IL-6 and TNF-α. The results from this study have identified several key protein markers that will further our understanding of the molecular mechanisms underlying microglial activation as well as possible therapeutic intervention for neuroinflammation using epigenetic modulators.

1224 Assessment of Microcystin's Neurotoxicity Using Two Knockout Strains of the Alternative Model *Caenorhabditis elegans*

C. Moore and B. Puschner. *Department of Molecular Biosciences, University of California-Davis School of Veterinary Medicine, Davis, CA*.

Microcystins (MCs) are well known acute lethal hepatotoxins produced by cyanobacteria worldwide, however, studies examining the potential non-lethal effects of MCs suggest that MCs are neurotoxic. MCs inhibit serine/threonine protein phosphatases (PPs) and therefore may alter neuron function. An alternative model using *Caenorhabditis elegans* (*C. elgans*) was developed based on the chemotaxis behavoir of *C. elegans* to diacetyl and benzaldehyde, which are detected by sensory neurons AWA and AWC. Models were evaluated using a binomial regression with quasibinomial error and showed that MC-LR and MC-LF alter AWA, but not AWC function in N2 (wildtype) nematodes with a non-monotonic dose response. To further investigate the targeted response of MCs on the AWA, changes in PP gene expression in N2 worms exposed to MC-LR and MC-LF were tested, along with chemotaxis of two knockout strains, CX2065 (AWC knockout) and CX32 (AWA knockout) exposed to 0, 1, 10, 50, 100, 150, 300, 500, or 1000 g/L MC-LR or 0, 1, 10, 50, 100, 150, or 300 g/L MC-LF. Preliminary data suggest that MC-LR alters AWA function in CX2065 at concentrations up to 300 g/L, while AWC function was unchanged. In CX32, preliminary results indicate that MC-LR and MC-LF alter AWC function at concentrations up to 300 g/L, while AWA function was unchanged. These data suggest that AWC neuron function is not required to observe MC neurotoxicity targeting the AWA neuron. In contrast, lack of AWA function may lead to MC-induced changes in benzaldehyde chemotaxis. Overall, both knock out strains performed well in the assay, without development of cytotoxicity at exposures up to 1000 g/L MC-LR. Additional, preliminary results indicate no significant changes in gene expression for PP1, PP2A, PP2B, PP4, PP6. These results will contribute to a better understanding of MC neuron specific neurotoxicity and the role of PPs in MC neurotoxicity. Support: US EPA (STAR FP917356 predoctoral fellowship for CEM), ACT North American Graduate Fellowship.

1225 Use of Stem Cell Derived Peripheral Neurons as a Model of Chemotherapeutic Induced Peripheral Neuropathy

S. Delaney[2], C. Wing[2], N. Favre[2], G. Luerman[1], E. Dolan[2] and B. Murphy[1]. [1]*Axiogenesis Inc, Plymouth Meeting, PA and* [2]*University of Chicago, Chicago, IL*. Sponsor: E. Clarke.

Chemotherapy induced peripheral neuropathy (CIPN) is one of the most common and potentially permanent side effects of chemotherapy, second only in frequency to hematopoietic toxicity. About 20-40% of cancer patients with estimates of about 400,000 patients each year develop CIPN as a consequence of treatment with platinum analogues (cisplatin, oxaliplatin, carboplatin), taxanes (paclitaxel, docetaxel), vinca alkaloids (vincristine), thalidomide, epothilones and bortezomib. Moreover, CIPN can lead to dose reduction of the chemotherapeutic agent or possible cessation of treatment. This may have an adverse impact on cancer treatment and disease outcomes. In this study, we evaluate the utility of induced pluripotent stem cell-derived human peripheral neurons as a cellular model to assist in the development of neuroprotective agents and validate genes thought to contribute to CIPN. Upon treatment of human peripheral neurons with paclitaxel, vincristine, bortezomib and cisplatin (1nM to 10 uM) up to 72 hours, we measured: 1) morphological characteristics such as relative outgrowth, process length, branches, and outgrowth intensity, 2) effects on spontaneous electrical activity by microelectrode array analysis, and 3) cell health as measured by Cell titer Glo. We identified reproducible decreases in relative neurite length and electrical spiking parameters per magnitude of drug concentration. The morphological changes differed across drugs, with vincristine and bortezomib treatment showing the most dramatic effect on neurite outgrowth, paclitaxel showing an intermediate effect, and cisplatin show-

ing no effect up to 10uM of drug. These cells provide a novel human peripheral neuron screening strategy and innovative means to evaluate neurotoxic chemotherapeutic agents with the ultimate goal of identifying new drugs to overcome this devastating side effect of chemotherapy.

1226 Acute Hydrogen Sulfide Exposure Induces Neurodegeneration via Activation of Inflammasomes in Brain

D.-S. Kim[3], P. Anantharam[3], E. M. Whitley[2], E. Gilbreath[1], B. Mahama[3] and W. K. Rumbeiha[3]. [1]*Pathobiology, School of Veterinary Medicine, Tuskegee University, Tuskegee, AL;* [2]*Pathogenesis, LLC, Gainesville, FL and* [3]*Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA.*

Hydrogen sulfide (H2S) is produced endogenously and has emerged as important gaseous signaling molecule. However, acute exposure to high concentrations of H2S induces brain damage, including long-term neurological dysfunction. To better understand the exact cellular and molecular mechanisms of H2S-induced neurological disorders, we investigated neuroinflammation aspect of H2S-induced toxicity using the Qiagen mouse Inflammation Response & Autoimmunity RT2 Profiler PCR array system. C57 black mice were exposed to 310 ppm H2S by whole body inhalation for 1, 3 or 7 days for 40 minutes on day 1 and 15 minutes on subsequent days. Hydrogen sulfide-exposed mice showed significant impairment in motor activity compared to the control. Histopathology revealed neurodegenerative lesions in the central nucleus of the inferior colliculus (IC), and IC was dissected at each terminal endpoint. Quantitative real-time PCR based array system was performed, and 84 inflammation related genes expressions were analyzed. Interestingly, we found the upregulation of IL-18 and Myd88 in day 3 and 7 H2S-treated groups compared to control. As IL-18 and Myd88 both play important roles in activation of inflammasomes, we further investigated genes relevant to the activation of inflammasomes using quantitative RT-PCR. IL-1β, CASP-1 and AIM2 were upregulated in H2S-treated groups. Expression of NLRP3 was downregulated, whereas NLRC4 was not greatly altered in H2S-treated groups. We also found that NLRP1b was upregulated in H2S treated group, while we are characterizing further the activation of inflammasomes and its significance in H2S-induced neurodegeneration. Taken together, our results demonstrated that inhalation exposure to H2S-induced motor behavioral deficit and neurodegeneration and that inflammasomes may play a significant role in H2S-associated neurological disorders (Iowa State Internal Grant).

1227 Small Molecule OT-404 Inhibits Oxidative Stress (OS) and NFκB Providing Protection in Cisplatin-Induced Peripheral Neuropathy (CIPN)

R. Alobaidi[1,2], S. Thangirala[1], S. A. Mousa[1] and H. A. El-Fawal[2]. [1]*Pharmaceutical Research Institute, Rensselaer, NY and* [2]*Neurotoxicology Laboratory, Albany College of Pharmacy and Health Sciences, Albany, NY.*

Peripheral neuropathy due to many diverse chemotherapeutics is a serious complication and limitation to effective cancer therapy. Several, but equivocal, mechanisms have been suggested for CIPN development, including OS and inflammatory amplification. OT-404, a Tempol congener, effectively inhibits cancer cell proliferation, tumor angiogenesis and improves the efficacy of traditional chemotherapeutics. In the present study the mechanisms of OT-404 actions were elucidated using NFκB transfected HeLa cells (104 cells/well), where 4μg/100μl OT-404 significantly ($p<0.05$) inhibited 100 ng/100μl cisplatin-induced NFκB activity, as determined by luciferase assay. OT-404 also prevented the translocation of p65, an NFκB active subunit, to the nucleus of THP-1 cells in response to LPS, as shown by Western blot. TNF-α production was also significantly ($p<0.01$) inhibited by OT-404 in the same cell line, while confocal microscopy of immunostained HUVEC cells showed that OT-404 also protected against 100 ng/ml TNF-α-induced NFκB activation. The ability of OT-404 (50μM) to reduce OS was demonstrated against H2O2 (300μM) by fluorescence in retinal ganglion cells (RGC5). The neuroprotective potential against CIPN was tested by daily injections of OT-404 at 30mg/kg in BALB/c mice receiving 1mg/kg cisplatin for 21 days (n=5-8/treatment). Hyperalgesia, determined by reduced tail flick latency, was significantly ($p<0.01$) attenuated by OT-404. Hind-limb escape extension was qualitatively decreased with cisplatin alone, but was comparable to controls for mice receiving cisplatin with OT-404. This neuroprotection was confirmed by toluidine blue-stained histopathology of sciatic nerves collected at 21 days. Nerves of cisplatin-treated mice showed thinning and loss of myelin with reduced axon caliber. These preliminary studies suggest that CIPN may be ameliorated by OT-404, possibly through the inhibition of OS, NFκB activity and inflammatory amplification, thus providing a potential adjuvant to chemotherapy.

1228 Development of an *In Vitro* Neuromuscular Junction System Between Human Stem Cell-Derived Motoneurons and Human Skeletal Muscle to Evaluate Compound Toxicity

N. Santhanam[3], L. Kumanchik[3], Y. Cai[3], M. Jackson[3], X. Guo[3], A. Lavado[3], C. W. McAleer[3], Y. Wang[3], M. T. Schnepper[3], C. Martin[3], F. Sommerhage[3], C. J. Long[3], A. Riu[1], J. Langer[2], L. Breton[1] and J. J. Hickman[3]. [1]*L'Oreal Research and Innovation division, Aulnay-sous-Bois, France;* [2]*L'Oreal Research and Innovation division, Clark, NJ and* [3]*NanoScience Technology Center, University of Central Florida, Orlando, FL.* Sponsor: E. Dufour.

The ability to efficiently monitor the functionality of neuromuscular junctions (NMJs) *in vitro* is essential to understanding the mechanisms that lead to their disruption during the early onset of neurodegenerative diseases and after exposure to toxins. Functional human- based *in vitro* NMJ systems eliminate the issue of species variability in the development of new therapeutics [1]. In this study we report a compartmentalized co-culture system that monitors the contractile functionality of neuromuscular interactions in response to isolated electrical stimulation of motoneurons. Motoneuron cell bodies were located on an array of microelectrodes in a motoneuron chamber while their axons extend through microfluidic tunnels to a distal chamber containing skeletal muscle on surface-modified microfabricated silicon cantilevers. Drugs that have been shown to compromise NMJ function were evaluated with the system. Two-chambered microfluidic devices have been used extensively to study neuronal function, however, to the best of our knowledge, this is the first time that such a platform has been coupled with two functional aspects namely, multi-electrode stimulation/recording and multi-cantilever deflection measurement. This system allows non-invasive stimulation of motoneuron cell bodies while monitoring the concurrent muscle-contraction initiated only through the formation of active neuromuscular synapses. While MEA systems monitor the electrophysiology of NMJs, this MECA system allows force measurement based on cantilever deflection of innervated muscle. This represents a next generation *in vitro* system for drug and toxin evaluation. [1] Guo et al. Biomaterials 32:9602-9611, 2011.

1229 Persistent Neuroinflammation in a Mouse Model of Tetramethylenedisulfotetramine (TETS)-Induced Status Epilepticus

C. A. Boosalis[3], D. Zolkowska[1], D. Bruun[3], J. Silverman[1], M. Rogawski[1] and P. Lein[3]. [1]*School of Medicine, Neurology, University of California-Davis, Sacramento, CA;* [2]*School of Medicine, Psychiatry, University of California-Davis, Sacramento, CA and* [3]*School of Veterinary Medicine, Molecular Biosciences, University of California-Davis, Davis, CA.* Sponsor: P. Lein.

TETS is a potent convulsant rodenticide that is considered a credible chemical threat agent. Humans exposed to TETS at high doses can exhibit acute seizures, status epilepticus (SE) and death. Survivors of TETS-induced SE are reported to exhibit spontaneous recurrent seizures and behavioral deficits. Emerging evidence suggests that persistent neuroinflammation contributes to the pathogenesis of epilepsy and to decrements in cognitive, psychomotor and affective behaviors; therefore, in this study we assessed neuroinflammatory responses and various behaviors in a mouse model of TETS-induced SE. Adult male NIH Swiss mice were injected with riluzole (10 mg/kg, ip) 10 min prior to injection with TETS (0.2 mg/kg, ip). TETS-intoxicated animals pretreated with riluzole exhibited >1 h of continuous seizure activity as assessed by electroencephalography and behavioral observation. In the absence of rescue therapy, ~90% of the animals died by 24 h post-TETS exposure. Diazepam (5 mg/kg, ip) or midazolam (0.73 mg/kg, im) administered 40 min after the initiation of seizure activity increased survival at 24 h post-TETS to 100% or > 75%, respectively. However, TETS intoxicated animals rescued by diazepam or midazolam exhibited significant region- and time-dependent reactive astrogliosis and microglial activation as determined by GFAP and Iba-1 immunoreactivity. Rescued animals also demonstrated increased anxiety-like behaviors in 1 of 2 cohorts tested and exhibited acoustic response deficits in 1 cohort. Together,

these studies identify a mouse model that will allow us to further assess the efficacy of candidate therapeutics for stopping TETS-induced SE and protecting against delayed and persistent neurological sequelae. This work is supported by the NINDS CounterACT Program (grant U54 NSS079202).

1230 Circadian Rhythm Disturbances Altered Learning, Response Inhibition, and Cholinergic Signaling in Long-Evans Rats

P. Eubig[1], R. Balachandran[1], M. Leventhal[1], K. Duenas[1], A. Robertson[1], S. Beaudin[2] and M. Mahoney[1]. [1]*Comparative Biosciences, University of Illinois, Urbana, IL and* [2]*Microbiology and Environmental Toxicology, University of California, Santa Cruz, CA.*

Biological rhythms are essential for physiology. Similar to other environmental risk factors such as stress, disruption to daily rhythms is associated with many health disorders, e.g., cancer, obesity, mood disorders. Yet, few studies have examined basic cognitive deficits caused by altered circadian rhythms. Here we examine how a shift work-like schedule of circadian disruption, which models a common form of rhythm disturbance, impacts attention, cognitive flexibility, and cholinergic signaling. Adult Long-Evans rats of both sexes were behavior tested either 4 hours after vivarium lights-on or lights-off. The 5-choice serial reaction time task (5CSRTT) and an operant set-shifting task were used to evaluate attention and cognitive flexibility, respectively. After the completion of testing, choline acetyltransferase (ChAT)-positive neurons were quantified in the nucleus basalis magnocellularis (NBM). Cholinergic neurons projecting from the NBM to the prefrontal cortex are particularly important for attentional performance and for learning, memory, and arousal. Night-tested rats made less correct responses and more premature responses on the 5CSRTT than day-tested rats, suggesting the primary impact on night-tested rats was on response inhibition, i.e., they were more impulsive, rather than on attention. Night-tested rats also took longer to meet criteria for the initial visual cue discrimination phase of set shifting, but did not differ from day-tested rats in the strategy-switching and reversal-learning phases, suggesting a difference in initial task acquisition but no differences in the cognitive flexibility components mediated by higher brain centers. NBM revealed night-tested rats had less ChAT-positive neurons than day-tested rats. Overall, these findings suggest that alterations of daily rhythms can affect response inhibition and the learning of simple behavioral response strategies, and may, in part, be mediated by cholinergic signaling.

1231 *In Vitro* Neurotoxicity of Different Tricresyl Phosphate (TCP) Isomers and Mixtures

D. J. Duarte, J. M. Rutten, M. van den Berg and R. H. Westerink. *Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands.*

Tricresyl phosphates (TCPs) consist of different isomers and mixtures thereof. In particular ortho-isomers, like tri-ortho-cresyl phosphate (ToCP), are well-known neurotoxicants. Exposure to TCPs, e.g. via contaminated cabin air, has been associated with health effects including the so-called aerotoxic syndrome. However, levels of ToCP in commercial TCP mixtures are very low, and recent risk and exposure assessments indicate that the reported health effects are unlikely to be due to ToCP exposure. However, the neurotoxicity of non-ortho isomers is largely unknown. With this research we therefore measured effects of exposure (up to 48h) to different (non-ortho) TCP isomers, commercial TCP mixtures and the metabolite of ToCP (CBDP: cresyl saligenin phosphate) on cell viability and mitochondrial activity, spontaneous neuronal electrical activity, and neurite outgrowth in primary rat cortical neurons. The results demonstrate that exposure to TCPs (24-48h, up to 10 μM) does not affect cell viability, the number of neurites per cell or the average neurite length, except for TmCP which induced a reduction of average neurite length. However, all TCPs increased mitochondrial activity already at 0.1-1 μM. Moreover, electrical activity is markedly decreased for the majority of TCPs (10 μM) following 48h exposure. Our combined data demonstrate that the different TCPs, including ToCP, are roughly equipotent for the studied endpoints. Notably, the no-observed-effect-concentrations from our study (0.1-1 μM) are well above current exposure levels, indicating limited neurotoxic health risk. However, exposures may have been higher in the past and our data indicate that prolonged and/or repeated exposure to TCPs may exacerbate the effects, which argues for additional research. Funding: Dutch Ministry of Infrastructure and the Environment (grant number 31099651) and the Faculty of Veterinary Medicine (Utrecht University).

1232 Neonatal Interleukin-1β Exposure Induces Chronic Neuroinflammatory Responses and Brain Damage in the Adult Rat

S. Lu[1], L.-T. Tien[3], Y. Pang[1], J. W. Lee[1], S. Lin[1], T. K. Jones[1,2], A. J. Bhatt[1], R. D. Savich[1] and L.-W. Fan[1]. [1]*Department of Pediatrics, Division of Newborn Medicine, University of Mississippi Medical Center, Jackson, MS;* [2]*HHMI Scholar Program, Tougaloo College, Tougaloo, MS and* [3]*School of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan.*

Our previous studies have shown that neonatal exposure to interleukin-1β (IL-1β) resulted in motor dysfunction and dopaminergic neuronal injury in the juvenile rat brain. The current study was designed to test whether such adverse effects could prolong into adult age. Sprague-Dawley male rat pups at postnatal day 5 (P5) were treated with IL-1β (1 μg/kg, intracerebral injection), motor behaviors were tested on P7 and P70, and brain injury were examined at P70. The results showed that neonatal IL-1β exposure resulted in disturbances of motor behaviors, including hyperactivity in the open field task, and dysfunction in the vibrissa-elicited forelimb-placing, movement initiation, pole and tapered/ledged beam walking tests. By P70, no significant differences in the above behavioral tests were observed between the IL-1β-treated and the control groups. However, IL-1β exposed group showed sustained neuroinflammatory responses, indicated by an increased number of activated microglia and elevated IL-1β and IL-6 contents in the brain. IL-1β exposure also led to a long-lasting injury to dopaminergic system, as evidenced by reduced tyrosine hydroxylase immunoreactivity in the substantia nigra. These results indicate that although neonatal IL-1β-induced neurobehavioral impairment is spontaneously recoverable, brain inflammation and damage to the dopaminergic system is prolonged into adult age, suggesting that IL-1β may play an important role in brain injury induced by perinatal infection/inflammation.

1233 Serotonin Reuptake Inhibitory Effects of Illegal Compounds Adulterated in the Anti-Obesity Food

S. H. Jung[2], Y.-J. Choi[2], K.-W. Kang[2], K. Shin[1] and B.-H. Lee[2]. [1]*Catholic University, Bucheon, Korea, Republic of and* [2]*Seuol National University, Seoul, Korea, Republic of.*

The serotonin transporter (SERT) is a type of monoamine transporter protein that transports serotonin from the synaptic cleft to the presynaptic neuron. This protein is the target of many antidepressant medications, such as sibutramine and fenfluramine. Analogues of fenfluramine and sibutramine, including n-nitroso fenfluramine, benzyl-, chloro-sibutramine are detected from dietary supplements as an illegal adulterant. However, serotonin reuptake inhibitory effects of these analogues were undeclared. In this study, Human embryonic kidney 293 cells (HEK-293) were stably transfected with corresponding cDNA of SERT (HEK-SERT). We examined the inhibition of serotonin reuptake on HEK-SERT following exposure to sibutramine, fenfluramine and illegal compounds (analogues of sibutramine and fenfluramine) by neurotransmitter transporter uptake assay. It was shown that both sibutramine and fenfluramine inhibited the serotonin reuptake. Also, three analogues of sibutramine (benzyl-, homo-, desmethyl-sibutramine) inhibited the serotonin reuptake. However, an analogue of fenfluramine (n-nitroso fenfluramine) did not influence the inhibition of serotonin reuptake. These results may help to clarify the serotonin reuptake inhibitory effect of illegal compounds adulterated in the anti-obesity food. This work was supported by a grant from ministry of food and drug safety.

1234 The Effect of Acute Acetaminophen Overdose on Hearing: Auditory Brainstem Responses, Protein Binding and Oxidative Stress in Cochleae

M. R. McGill[4], S. Kennon-McGill[4], D. Durham[3] and H. Jaeschke[3]. [1]*Otolaryngology - Head and Neck Surgery, University of Kansas Medical Center, Kansas City, KS;* [2]*Pathology and Immunology, Washington University School of Medicine, St. Louis, MO;* [3]*Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS and* [4]*Psychiatry, Washington University School of Medicine, St. Louis, MO.*

The liver is the primary target organ in acetaminophen (APAP) toxicity, but other organs are affected. Previous data have shown that chronic APAP/opioid abuse can be ototoxic. *In vitro* studies revealed that APAP is likely the ototoxic agent and the mechanism involves APAP-induced oxidative stress and reactive metabolite (NAPQI)-induced endoplasmic reticulum stress. However, the effect of a single acute overdose on hear-

ing has not been tested, nor have any *in vivo* studies been performed. We sought to determine if acute APAP overdose causes hearing damage *in vivo*, and to explore possible mechanisms of APAP ototoxicity. Male C57BL/6J mice were treated with a single human-relevant overdose of APAP (300 mg/kg). Blood, liver and cochleae were harvested at 0, 2, 6 and 24h post-APAP. In some mice, auditory brainstem responses (ABRs) to a range of frequencies were measured at 24h. The furosemide plus kanamycin (FS/K) model of drug ototoxicity was used as a positive control for hearing loss. NAPQI formation after APAP was assessed by measuring glutathione (GSH) depletion and covalent protein binding, and oxidative stress was assessed by measuring glutathione disulfide (GSSG). There was no evidence of reactive metabolite formation or hearing loss after a single clinically relevant overdose of APAP. However, there was a transient increase in oxidative stress. The latter may support a role for oxidative stress in hearing loss due to chronic APAP abuse. Overall, a single human-relevant acute overdose of acetaminophen (APAP) caused transient oxidative stress in cochleae but not hearing loss.

PS 1235 Acute Acetaminophen (APAP) Intoxication Decreases Locomotor Activity and Modifies Glial Cells Markers in Brain Areas Involved in the Locomotion and Memory Regulation

M. B. Vigo[3], M. J. Peréz[4], E. Casim[3], M. B. Di Carlo[2], A. Scazziota[2], S. Martinez[1], J. E. Manautou[6] and C. I. Ghanem[3,5]. *[1]3er UA, Departamento de Biología Celular, Histología, Embriología y Genética, Facultad de Medicina, UBA, Bs As, Argentina; [2]Dpto. Bioquimica Clinica, FFyB, UBA, Bs As, Argentina; [3]Instituto de Investigaciones Farmacológicas (ININFA). CONICET-UBA, Bs As, Argentina; [4]Instituto de Química y Fisicoquímica Biológica. (IQUIFIB). CONICET, Bs As, Argentina; [5]Cátedra de Fisiopatología, Facultad de Farmacia y Bioquímica. UBA, Bs As, Argentina and [6]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT.*

Brain damage after APAP poisoning is usually associated with encephalopaty, secondary to acute liver failure (ALF). APAP also produces astrogliosis and death of oligodendrocyte precursor cells (OPC) *in vitro*. The aim of this work was to evaluate the effect of acute APAP intoxication, in the absence of ALF, on locomotor activity and glial cell markers in pre-frontal cortex (Pfc), nucleus accumbesns (Ac), striatum (St) and hippocampus (Hp). Male Wistar rats were dosed with APAP (1g/kg) or vehicle. 24h later, markers of hepatic necrosis (ALT/AST) and liver functional markers (KPTT, TP, Factor V and plasma ammonia levels) were evaluated. Locomotor activity was measured using the Ethovision tracking system. The protein expression of the astrocyte marker Gfap and markers of oligodendrocyte differentiation (PdgfR-a, SOX10 and MBP) were also evaluated in St, Ac, Pfc and Hp. AST and ALT values were increased by 200% and 110%, respectively by APAP, compared to controls. In contrast, functional liver markers KPTT, TP, FV and ammonia levels were not changed. However, locomotor activity was 53 % lower in APAP treated rats than in controls. Gfap was significantly increased by 8%, 202%, 70% and 199% in Pfc, Ac, St and Hp, respectively, while the expression of SOX 10, a marker of oligodendrocyte lineage, was not changed by APAP. However, the OPC marker PdgfR-a was increased in Es and Hp by 148 % and 162%, respectively, with no changes in Pfc and Ac. Lastly, the marker of differentiated oligodrendocytes, MBP, decreased in Hp by 49% but not in Pfc, Ac and Es. In conclusion, these data indicate that acute APAP intoxication in rats produces changes in nerve conduction and direct toxicity to glial cells in the absence of ALF. The main features are astrogliosis and a decreased in number of mature oligodendrocytes and OPC proliferation.

PS 1236 Neonatal Exposure to Paracetamol (Acetaminophen) and CB1R Agonist Show an Additive Adverse Neurodevelopmental Effect

G. Philippot, A. Fredriksson and H. Viberg. *Environmental Toxicology, Uppsala University, Uppsala, Sweden.*

Among pregnant women, newborns and toddlers, paracetamol is one of the most used analgesic and antipyretic drugs. Recent results show that exposure to paracetamol during brain development increases the risk for neurobehavioral deficits in humans and affects levels of neurotransmitters and adult cognitive function and behavior in rodents. In the present study, we continued our evaluation of the developmental neurotoxic effects of paracetamol, and since paracetamol metabolite AM404 has been shown to interact with cannabinoid receptor type 1 (CB1R), we hypothesize that the endocannabinoid system is involved in the paracetamol-induced neurotoxic effect. On postnatal day 10 we exposed male mice to paracetamol (dose = 30 mg/kg), CB1R agonist WIN55212-2 (WIN) (dose = 1mg/kg) or a combination of both. Expression of genes involved in brain development were evaluated 24 h after exposure. Spontaneous behavior, when introduced to a novel home environment, was tested at the age of 2 months. Decreased gene expression of tropomyosin receptor kinase B was observed for both paracetamol, WIN and combination groups. Decreased gene expression was also observed for genes important in glutamatergic, dopaminergic and endocannabinoid signaling, but only when both paracetamol and WIN were given together. Mice neonatally exposed to a double dose of paracetamol, 4 h apart, but not to a single dose of either paracetamol or WIN, showed altered adult spontaneous behavior and decreased habituation capability in a novel home environment. When single doses of paracetamol and WIN were given simultaneously, the same adverse effect was seen as for the double dose of paracetamol. These effects were even more significant when the double dose of paracetamol was combined with WIN. In summary, neonatal exposure to paracetamol and WIN show an additive adverse effect on gene expression and behavior in adult mice. The known interaction between AM404 and CB1R together with the present results suggest that the endocannabinoid system is involved in the neurotoxic effects of paracetamol.

PS 1237 Drug-Induced Neurotoxicity Assessments: Validation of a Neurological and Functional Observation Battery in the Sinclair Miniature Swine

A. Stricker-Krongrad[2], M. Zhong[2], A. Fuller[2], D. Brocksmith[1], J. Liu[2] and G. Bouchard[2]. *[1]Sinclair BioResources, Auxvasse, MO and [2]Sinclair Research Center, Auxvasse, MO.*

The nervous system is one of the most complex organs in the body, and has its unique vulnerabilities to toxic compounds. The central nervous system, along with cardiovascular and respiratory systems, has been listed in the safety pharmacology core battery of the harmonized test guidelines for preclinical testing of human pharmaceuticals (ICH S7A, 2000). A functional observational battery (FOB) has been recommended in the guideline as the first tier neurotoxicity screening that encompasses motor activity, behavioral changes, coordination, sensor/motor reflex responses, and body temperature. The miniature swine is an appropriate species for neurological and behavioral studies: its central nervous system and specifically the brain are similar to those of human with respect to tissue composition, gyrencephalic structure, and developmental growth and myelination patterns. In addition, behavioral studies, as well as modeling of neurological human diseases, have been increasingly performed in minipigs. Because the miniature swine has increasingly been used as an alternative to dog or non-human primate in regulatory toxicology studies, we wanted to validate a FOB for the safety pharmacology assessment of pharmaceutical products intended for human use. A swine FOB protocol was developed with multiple observation parameters to monitor effects on six majors categories related to sensory and motor functions, autonomic and voluntary functions and behavior. A crossover study was performed to capture the neurological effects of CNS acting agents administered to the Sinclair minipigs via intramuscular route. Amphetamine, ketamine and diazepam were demonstrated to positively or negatively dose-dependently affect different parameters related to autonomic functions, excitability, behavior, motor activities, gait and reflexes.

PS 1238 Evaluation of the Effects of Tanezumab on Sympathetic Ganglia in Cynomolgus Monkeys (*Macaca fascicularis*): Stereologic, Histomorphologic, and Cardiofunctional Assessments

P. Belanger[2], M. Butt[4], P. Butler[2], S. Bhatt[1], S. Foote[1], D. Shelton[3], M. Evans[2], R. Arends[1], S. Hurst[1], C. Okerberg[2], T. Cummings[1], D. Potter[1], J. Steidl-Nichols[1] and M. Zorbas[2]. *[1]Pfizer, Inc., Groton, CT; [2]Pfizer, Inc., San Diego, CA; [3]Pfizer, Inc., South San Francisco, CA and [4]Tox Path Specialists, Frederick, MD.*

Tanezumab, a humanized monoclonal antibody against nerve growth factor (NGF), is under development for treatment of chronic pain. In 2012, the US Food and Drug Administration placed a partial clinical hold on all NGF inhibitors due to adverse changes in the sympathetic nervous system (SNS) of mature animals. Three studies assessed tanezumab effects on the SNS of mature cynomolgus monkeys. Study 1 evaluated potential SNS effects of subcutaneous (SC) tanezumab (1.2 mg/kg/8 weeks) for 3 or 6 months and reversibility or persistence of any effects through a non-dosing/recovery period. Study 2 evaluated whether or not neuronal death occurs shortly (2-28 days) after a single SC tanezumab dose (1.2 mg/kg). The superior cervical ganglion (SCG) was evaluated for neuronal death and morphology, as well as ganglion volume, average neuron size/area and estimated total neuron counts via stere-

ology. Study 3 evaluated effects of SC tanezumab (1.2 mg/kg/8 weeks or 30 mg/kg/week) on SNS function over a 6-month period by assessing ambulatory cardiovascular parameters via telemetry and a tilt paradigm to assess orthostatic hypotension. There was no evidence of neuronal death at any timepoint following tanezumab treatment. At 1, 3 and 6 months, tanezumab exposure was associated with stereologic changes in the SCG, including smaller ganglion volume, smaller average neuron size/area, and/or lower estimated neuron counts with complete reversibility observed upon tanezumab withdrawal (indicating no actual loss of neurons). Tanezumab had no adverse effects on ambulatory cardiovascular parameters or group mean systolic blood pressure responses to orthostatic challenge. Supratherapeutic doses of tanezumab had no adverse effects on SNS morphology or function in mature monkeys.

PS 1239 Approach to Development of Simple Method for Detecting Rewarding Effect of Morphine Using Zebrafish

Y. Yoshizawa, I. Miyawaki, J. Deguchi and H. Funabashi. *Preclinical Research Laboratories, Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan.* Sponsor: T. Yamada.

Introduction: Recently, zebrafish (*Danio rerio*) has attracted interest as an experimental animal alternative to mammals and been used for drug development. In the present study, we attempted to detect the rewarding effect of morphine (Mor) in larval or adult zebrafish by the original method based on the conditioned place preference paradigm often used in rodents. Method: Larva: Zebrafish (14 days post fertilization) were put in 24-well red plate filled with MOR one each for 360-min as a conditioning, then were moved to another 24-well plate composed of red and white zone filled with water (Wat) for test session. Time spent in either zone was measured using a video tracking system for 5-min. Time spent in red zone was regarded as an index of rewarding effect. Adult: Zebrafish (>3 months old) were put in the rectangular tank composed of 2 visually distinct compartments (black dots and no dots) separated by a central wall. Animals were alternately confined in either compartment with Wat or Mor overnight. This consecutive 2-day operation (Wat-Mor) was defined as 1 set of conditioning. After up to 4 sets of conditioning, zebrafish (N= 8 to 9/tank) were allowed to access freely to both compartments for 60-min for test session. The number of zebrafish in both sides was counted every 1-min using snapshots of video-recorded data. The difference of total number present in the Mor-side between before and after conditioning was regarded as an index of rewarding effect. Results and discussion: Larva showed a trend toward preference for Mor, but which was slight and no statistically significant. Adult showed a trend toward preference for Mor after 1 set of conditioning and a significant preference was observed after 4 sets of conditioning. This difference of responses between larva and adult could be due to the difference in neural development involved in memory or learning. We could demonstrate the rewarding effect of Mor in zebrafish using our method. The adult method could be especially useful because there's no need for long time or complex work.

PS 1240 Changes in Gene Expression After Prolonged Sevoflurane Exposure in Developing Monkeys

F. Liu, T. A. Patterson, M. G. Paule, W. Slikker and C. Wang. *National Center for Toxicological Research, Jefferson, AR.*

Sevoflurane is a widely used volatile anesthetic in general anesthesia, yet its safety in pediatric use is of public concern. The present study sought to evaluate whether prolonged exposure of infant monkeys to a clinically-relevant concentration of sevoflurane is associated with any adverse effects on the developing brain using DNA microarray analysis. Infant monkeys were exposed to 2.5% sevoflurane for 9 hours, and the brain tissues in frontal cortical levels were harvested for RNA extraction. Gene expression profiling was performed using the Agilent M. Mulatta (Rhesus) Oligo Microarray platform containing 43663 probes. The gene expression data from 4 sevoflurane-exposed and 4 control monkeys were analyzed using GeneSpring GX with quantile normalization. Using the inclusion criteria of greater than a 1.5 fold-change in expression (either up or down) and a p-value<0.05, a list of 576 DEGs was identified: 303 genes were up-regulated and 273 genes were down-regulated in the sevoflurane-exposed monkey brains. These genes were loaded into Ingenuity Pathway Analysis database for pathway, disease and biological function, and network analyses. The results indicated that the DEGs contributed significantly to nervous system development and functions; neurological diseases; cell death and survival and cell cycling. Moreover, a considerable number of DEGs were associated with networks of lipid metabolism, molecular transport and small molecule biochemistry. Findings from the microarray analysis have provided potential to further evaluate the mechanisms that underlie sevoflurane-induced neural damage during development (Supported by NCTR/US FDA and CDER/US FDA).

PS 1241 Sevoflurane-Induced Developmental Neurotoxicity and Potential Biomarkers

W. Slikker[2], F. Liu[2], X. Han[1], T. A. Patterson[2], M. G. Paule[2] and C. Wang[2]. [1]*Diabetes and Obesity Research Center, Sanford-Burham Medical Research Institute, Lake Nona, FL and* [2]*Division of Neurotoxicology, NCTR/US FDA, Jefferson, AR;* [3]*Office of Regulatory Compliance and Risk Management, NCTR/US FDA, Jefferson, AR;* [4]*Office of the Director, NCTR/US FDA, Jefferson, AR.*

Sevoflurane is a volatile anesthetic widely used in pediatric general anesthesia. The present study evaluated whether a prolonged exposure of monkey infants to a clinically-relevant concentration of sevoflurane produced any adverse effects on the developing brain. Postnatal day (PND) 5 or 6 rhesus monkeys were exposed to 2.5% sevoflurane for 9 hours and frontal cortical brain tissue was collected for lipidomic, luminex protein and histological assays. Lipidomic analyses demonstrated that critical cellular lipid components, e.g., phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylglycerol (PG) were significantly decreased, whereas 4-hydroxynonenal (HNE) was significantly increased after prolonged sevoflurane exposure compared with controls. Consistent with the lipid alterations, luminex protein analysis indicated abnormal levels of several cytokines in sevoflurane-exposed brains. Consistently, Fluoro-Jade C staining demonstrated more degenerating neurons after sevoflurane exposure. These data demonstrate that a clinically-relevant concentration of sevoflurane (2.5%) can induce and maintain an effective surgical plane of anesthesia in the developing nonhuman primate, and prolonged sevoflurane exposure produces significant changes in cytokine levels, lipid metabolism and neuronal damage. Along with alterations in cytokine levels, sevoflurane-induced neuronal damage appears to be effectively reflected in changes in brain lipid composition and/or content. Specific lipid changes may be sensitive biomarkers for the early detection of anesthetic-induced perturbation of the developing nervous system. (Supported by NCTR/US FDA and CDER/US FDA).

PS 1242 Relationship Between Ketamine-Induced Toxicity and NMDA Receptor-Mediated Calcium Influx in Developing Neurons

C. Wang, F. Liu, T. A. Patterson, M. G. Paule and W. Slikker. *National Center for Toxicological Research, Jefferson, AR.*

Ketamine, a noncompetitive NMDA receptor antagonist, is used as a general anesthetic and recent data suggest that anesthetics can cause neuronal damage when exposure occurs during development. To elucidate the underlying mechanisms associated with ketamine neurotoxicity, neural cells were harvested from the forebrain of newborn rats and neural stem cells were isolated from gestational day 16 rats. To determine the effect of ketamine on developing neurons and undifferentiated neural stem cells, cultures were exposed to 10 μM ketamine for 24 hours. Ketamine exposure resulted in elevated NMDA receptor (NR1) expression in primary cultures, and enhanced damage of developing neurons including those differentiated from the neural stem cells. However, the viability and proliferation rate of neural stem cells were not significantly affected after ketamine exposure. Since NMDA receptor-regulated ion channels are known to be highly permeable to calcium, a study to monitor the presence of, and/or changes in, intracellular calcium concentrations would be informative. Calcium imaging data indicated that 50 μM NMDA did not cause a significant influx of calcium in typical neural stem cells; however, it did produce an immediate elevation of intracellular free Ca2+ [Ca2+]i in neurons differentiated from the same neural stem cells. NMDA (50 μM) also produced an elevation in [Ca2+]i in both control and ketamine-exposed neurons in the primary cell cultures, and a significant increase in [Ca2+]i was detected in ketamine-exposed neurons compared with control neurons after NMDA stimulation. These findings suggest that prolonged exposure of developing neurons to ketamine produces an increase in NMDA receptor expression (compensatory up-regulation) which allows for a higher/toxic influx of calcium into neurons once ketamine is removed from the system, leading to neuronal cell death likely due to elevated reactive oxygen species generation. The absence of functional NMDA receptors in cultured neural stem cells may help to explain why clinically-relevant concentrations of ketamine did not affect undifferentiated neural stem cell viability. Supported by NCTR/US FDA and CDER/US FDA (E-7494)

1243 Serotonin 5HT2 Receptors Antagonism Prevents the Development and Expression of Ketamine-Induced Behavioral Sensitization in Mice

Y. Abreu-Villaca[1,2], J. P. Galvanho[1], A. C. Manhaes[1] and C. C. Filgueiras[1]. [1]*Ciencias Fisiologicas, UERJ, Rio de Janeiro, Brazil and* [2]*Psychiatry & Behavioral Sciences, Duke University Medical Center, Durham, NC.* Sponsor: E. Levin.

The serotonergic system has been suggested to interact with drugs of abuse. However, its effects seem to vary depending on the drug so that this system was shown to play an important role in the psychomotor stimulant response to cocaine but not to phencyclidine. Ketamine use as a drug club is increasing worldwide. It is a classic N-methyl-D-aspartate receptor antagonist, however, there is evidence that it also acts as a 5HT2 receptor agonist. Accordingly, the objective of the current study was to evaluate the acute and repeated effects of a subanesthetic dose of ketamine in a rodent model of behavioral sensitization and whether ketanserin, a 5HT2 antagonist, interferes with its development and/or expression. Adult male Swiss mice (8-10/group) habituated to an open field for 20 min during 3 days. Subsequently, for 7 consecutive days (acquisition period), the animals either received saline (Veh) or ketanserin (Ks, 3mg/kg, s.c.). Ten minutes later, the animals received saline or ketamine (Ket, 30mg/kg, i.p.) and their locomotor activity was assessed for 20 min in an open field. Accordingly, at this point, there were four groups: Veh, Ket, Ks and Ks-Ket. After a five-day withdrawal period, these groups were subdivided to receive saline or a ketamine challenge. The effect of this challenge was investigated in a sensitization test. ANOVAs followed by posthoc Duncan tests indicated that while the Ket group presented the higher levels of locomotor activity during the acquisition period, pre-exposure to ketanserin prevented this effect. During the sensitization test, the challenge with ketamine produced higher levels of activity in the ket group. In addition, ketanserin administration during the acquisition phase was able to reduce the response to ketamine even after five days of withdrawal. The inhibition of ketamine-induced hyperactivity by ketanserin during the acquisition period indicates that this 5HT2 antagonist prevented the development of behavioral sensitization. A similar effect during the sensitization test suggests that ketanserin was also able to inhibit the expression of behavioral sensitization.

1244 Concurrent Inhibition of Vesicular Monoamine Transporter 2 Does Not Protect Against 3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) Induced Neurotoxicity

T. J. Monks, A. B. Cholanians, L. L. Lizarraga, A. V. Phan and S. S. Lau. *Pharmacology and Toxicology, The University of Arizona, Tucson, AZ.*

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) is a hallucinogenic amphetamine derivative, with high abuse potential. The initial acute effects of MDMA are hyperthermia, hyperactivity and behavioral changes, which are followed by long term-neurotoxicity. The long-term neurotoxicity manifests as serotonergic axonal degeneration, depletion of indolamines, and loss of serotonin reuptake transporters in rats and primates, including humans. However, the underlying mechanism of neurotoxicity remains elusive. We have shown that pre-treatment of rats with R0 4-1284 (R0), a reversible vesicular monoamine transporter 2 (VMAT2) inhibitor, reduced hyperactivity in rats, and abolished the hyperthermic response and long-term neurotoxicity. The current study focused on protective effects of co- and/or post-inhibition of VMAT2 following MDMA administration. After recovery from implantation of Subcue® body temperature (T°) dataloggers, Sprague Dawley rats received MDMA (20, 25, or 27.5 mg/kg s.c.), with co- and/or post-treatment with R0 (10 mg/kg i.p.). One week after treatment, animals were euthanized and brains microdissected for indolamine analysis. Rats simultaneously treated with R0 and MDMA exhibited a more rapid increase in T°compared to animals treated with just MDMA. However, the persistence of the elevated T° in co-treated animals was significantly shortened compared to rats only treated with MDMA (~3hrs vs 8 hrs, respectively). A similar T° response was observed in rats post-treated (7 hours after MDMA) with R0 compared to animals only receiving the co-treatment. Despite decreases in AUC of T° with the R0 treatments, there were no significant differences in the extent of indolamine depletion between MDMA treated rats and MDMA/R0 rats. The results suggest that the long-term neuroprotective effects of VMAT2 inhibition is more likely due to the indirect monoamine depleting effects of the R0 pre-treatment, rather than by the direct inhibition of VMAT2 function.

1245 High-Resolution Metabolomics of Anti-Seizure Therapy in Pregnant Women with Epilepsy

D. I. Walker[5], K. D. Pennell[1], R. H. Finnell[4], K. J. Meador[3], D. P. Jones[5] and P. B. Pennell[2]. [1]*Dept. of Civil and Environmental Engineering, Tufts University, Medford, MA;* [2]*Dept. of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA;* [3]*Dept. of Neurology, Stanford University, Stanford, CA;* [4]*Dept. of Nutritional Sciences and Chemistry, University of Texas at Austin, Austin, TX and* [5]*Division of Pulmonary, Allergy and Critical Care Medicine, Emory University, Atlanta, GA.*

Antiepileptic drugs (AEDs) are among the most frequently prescribed known teratogens during pregnancy, resulting in increased risk for congenital malformations and neurodevelopmental deficits. To investigate metabolic alterations occurring from AED treatment during pregnancy, we completed high-resolution metabolomic (HRM) profiling of 500 plasma samples collected from 130 pregnant women with epilepsy at trimesters 1-3 and delivery. HRM profiling of matched cord blood from 72 deliveries was also completed. Samples were analyzed using HILIC chromatography interfaced to a Thermo Q-Exactive HF high-resolution mass spectrometer, which we then tested for differential expression based on AED therapy at trimesters 1-3, delivery and within the neonatal cord blood. Comparison of the major frontline AEDs, lamotrigine (LTG) and levetiracetam (LEV), indicated drug specific metabolic profiles could be identified within the maternal plasma. For LTG monotherapy, both the parent and major metabolites, including LTG-2N-glucuronide, LTG-2N-methyl and LTG-2N-oxide were detected, while LEV and 2-pyrrolidone-N-butyric acid were detected in patients maintained on LEV. Matched maternal-neonatal cord blood correlation indicated a linear association with maternal levels of AED metabolites. Metabolic pathway enrichment suggested alterations in tyrosine ($p= 0.007$) and biopterin ($p= 0.009$) metabolism, important metabolic intermediates for synthesis of neurotransmitters. These results represent a first step in identifying the AED related metabolic changes occurring during pregnancy. Study supported by NIH award number 3UO1NS038455-13S1.

1246 Identification of Cochlear Hair Cell Injury After Treatment with Kanamycin and Furosemide in C57BL6 Mice

K. Tenneson and J.-F. Lafond. *Charles River, Senneville, QC, Canada.*

Background: A number of drug classes have been shown to be ototoxic, including antibiotics, loop diuretics, platinum-based chemotherapy agents and several nonsteroidal anti-inflammatory drugs (NSAIDS). The potential for sensorineural hearing loss, dysequilibrium, or both in patients has gradually resulted in increased interest in screening of new chemical entities during early drug development. We characterized an animal model of cochlear hair cell injury in the mouse, using two known ototoxic compounds, as a tool to screen for ototoxicity. Methods: Four groups of 4 C57BL6 female mice/group were administered a single dose of kanamycin (1 mg/g) by subcutaneous injection and furosemide (0.08, 0.10, 0.25 or 0.40 or mg/g) by intraperitoneal injection. Animals were monitored for 10 days. At necropsy, the tympanic bulla and inner ear were removed, and the tissue was fixed and decalcified using a solution of picric acid and formic acid (PFF). The inner ears were then step-sectioned at 75 µm intervals and stained with hematoxylin and eosin. Results: Following dose administration, transient reaction to treatment was observed at 0.40 and 0.25 mg/g furosemide, consisting of decreased activity, partly closed eyes and decreased respiratory rate. Microscopically, a generally dose-dependent increase in the incidence and severity of findings in the cochlea were observed in animals administered furosemide at ≥ 0.10 mg/g. Outer hair cells of the cochlea were the most susceptible to degenerative changes of kanamycin/furosemide, followed by, in a decreasing order of sensitivity, the inner hair cells, the supporting cells (i.e. pillar cells and/or phalangeal cells) and the stria vascularis. Conclusion: 10 days following systemic administration of kanamycin/furosemide to the C57BL6 mouse, ototocxicity was characterized by degeneration/loss of the outer and inner hair cells, degeneration of the supporting cells and degeneration/atrophy of the stria vascularis.

1247 Retigabine Avoids Behavioral Neurotoxicity Associated with Early Life Phenobarbital Exposure in Rats

S. Frankel, N. Medvedeva, S. B. Gutherz, C. V. Kulick, C. Soper, A. Kondratyev and P. A. Forcelli. *Dept of Pharmacology & Physiology, Georgetown University, Washington, DC.* Sponsor: R. Tucker.

Background: Recent evidence shows deficits in behavioral domains ranging from learning and memory to anxiety-like behavior in rats treated neonatally with anticonvulsant drugs, such as phenobarbital (PB). Moreover, even a single neonatal exposure to PB triggers profound neuronal apoptosis in the developing rat brain. Retigabine (RTG) is a new-generation anticonvulsant drug; chronic, but not acute exposure to RTG, induces neuronal cell death. Here, we examined the effects of neonatal RTG exposure on adult behavior. Methods: Sprague-Dawley pups were treated with either vehicle, PB (positive control, 75 mg/kg, ip) or RTG (30 mg/kg, ip) on postnatal day (P)7, 8, 9, 11, 12, and 13. Starting on P60, rats were tested on a battery of behavioral tests. Animals were first tested in an open field arena for locomotor activity, followed by the elevated plus maze (anxiety-like behavior) and the light-dark task (anxiety-like behavior). Performance on an accelerating rotorod was next examined, followed by social interaction, passive avoidance, and tail flick latency. Results: General sensory and motor ability: Neither RTG, nor PB exposure in early life impacted adult sensory or motor ability. Anxiety-like behavior: Early life PB, but not RTG, resulted in increased anxiety-like behavior in the elevated plus maze. Neither PB nor RTG significantly altered the number of light-dark transitions in the light-dark task, nor the total time in the light zone, but both treated groups displayed longer visits to the dark zone, consistent with increased anxiety-like behavior. Social behavior: While neither RTG nor PB differed significantly from vehicle-treated animals, these treatments did differ from each other, with PB animals displaying lower social interaction than RTG-treated animals. Learning and Memory: PB, but not RTG exposure impaired retention in the passive-avoidance task. Conclusions: These data are consistent with our prior reports regarding behavioral changes following early life phenobarbital exposure. They indicate that some, but not all behavioral changes triggered by PB (and other AEDs) are induced by RTG. RTG exposure increased anxiety behavior in one of two tasks, leaving learning and memory domains intact. This is consistent with the more benign profile of RTG as compared to PB with respect to induction of neuronal apoptosis in the neonatal rat brain.

1248 Time-Course Study on Marijuana-Induced Dyslipidemia in Brain of Rats

R. N. Ugbaja[2], O. Ademuyiwa[2], B. O. Onunkwor[2], O. A. Dosumu[2], D. Babayemi[2] and T. F. Akinhanmi[2]. *[1]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria and [2]Department of Chemistry, Federal University of Agriculture, Abeokuta, Nigeria.*

Marijuana is a substance virtually abused in all countries. It elicits its action through the brain. The brain is the body's control centre with different regions performing different functions. It is rich in lipids, which could be affected in marijuana use since it is lipophilic. The effects of marijuana extract on lipids in different regions of brain (medulla oblongata (MO), cerebellum (CB), right cerebrum (RC) and left cerebrum (LC)) were investigated. Male rats were administered marijuana extract (12.5, 25 and 50mg/kg body weight) and control rats were given olive oil daily for 2, 4 and 6 weeks respectively. Cholesterol level in MO was decreased (55%) by the 12.5mg dose at 6 weeks compared with control. While doses did not affect cholesterol level in RC, a constipation of cholesterol (127%) was observed at 6 weeks in LC. Cholesterol level was depleted (15%) at 6 weeks by the 50mg dose. A dose-dependent decrease in cholesterol level from 2nd to 6th week was observed in CB. Phospholipidosis was the hallmark of dyslipidemia in the MO by the 12.5 and 25 mg doses at 2 and 4 weeks compared with control. Phospholipid levels were decreased by 50mg dose at 4 weeks (19%) and 12.5mg dose at 6 weeks (16%) compared with control. In the RC, 12.5mg dose increased phospholipid level while 25 and 50mg doses decreased the level compared with control. At 6 weeks, 50 mg dose increased phospholipid level (79%) from the 4th week in RC. Phopholipidosis in CB by the different doses was at 2 weeks. At 4 weeks, the 12.5 and 50mg doses sustained this condition while the 25 and 50mg doses sustained it at 6 weeks. In LC, 12.5mg dose-dependently increased phospholipid level from 2 to 6 weeks while a dose-dependent decrease was elicited by the 50mg dose. These results indicate that marijuana could cause dyslipidemia in different regions of brain which could allow a compromise of the function of brain and in part explain some abnormalities observed in marijuana users.

1249 Time Course of Arsenic-Induced Lipotoxic and Non-Lipotoxic Dyslipidemia in Male Albino Rats: Comparison Between Pentavalent Inorganic and Organic Arsenic Through Drinking Water

O. Ademuyiwa[2], J. O. Fatoki[3], R. N. Ugbaja[2], T. F. Akinhanmi[2], O. K. Afolabi[3], O. O. Ogunrinola[4], A. D. Wusu[4], E. O. Abam[1], O. A. Dosumu[2], D. O. Babayemi[2] and O. B. Onunkwor[2]. *[1]Biochemistry, Bells University of Technology, Ota, Nigeria; [2]Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria; [3]Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Nigeria and [4]Biochemistry, Lagos State University, Lagos, Nigeria; [5]Chemistry, Federal University of Agriculture, Abeokuta, Nigeria.*

Arsenic exposure through drinking contaminated water is a significant risk factor in the development of cardiovascular diseases through an unknown mechanism. Over the years, epidemiological studies have also identified lipid and lipoprotein abnormalities as independent risk factors in the pathogenesis and progression of atherosclerosis and cardiovascular diseases. In order to investigate the time course of arsenic-induced perturbations in lipid metabolism, male albino rats (n=90) were exposed to arsenic (100ppm, 150ppm and 200ppm) for four (4) weeks, eight (8) weeks and twelve (12) weeks as sodium arsenate and dimethylarsinic acid (DMA) in their drinking water. Control animals (n=30) received distilled water for the same period after which blood, liver, brain and kidney were removed from the animals and analysed for lipid dynamics. The two arsenicals elicited both lipotoxic and non-lipotoxic effects in the animals. Lipotoxic effects in plasma were characterised by hypercholesterolemia and hyperphospholipidemia while erythrocyte lipotoxicity was characterised by phospholipidosis. In the HDL, increased phospholipid concentration was the hallmark of lipotoxicty. In the hepatic, renal as well as the brain compartments, enhanced cholesterogenesis and phospholipidosis were the hallmarks of lipotoxicty on exposure to the two arsenicals through drinking water. In contrast however, decreased reverse cholesterol transport and reduced erythrocyte cholesterol characterised the non-lipotoxic effects of the two arsenicals. Our findings indicate that arsenic up- or down-regulates different pathways in the lipid metabolism spectrum at "low" or "high" doses and this might be responsible for the cardiovascular and other disease endpoints of exposure to this metalloid.

1250 Low Level Arsenic Exposure Reveals Mitochondrial Dynamics Regulation of Self-Renewal

A. Cheikhi, F. Ambrosio and A. Barchowsky. *Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA; Physical Medicine and Rehabilitation, University of Pittsburgh, Pittsburgh, PA.*

We have reported that low level arsenic exposure disrupts skeletal muscle stem cell (MuSC) mitochondrial function and bioenergetics to compromise regenerative capacity and injury repair. The dysfunctional mitochondrial phenotype was observed in generations of MuSC after removal of arsenic, indicating epigenetic change. This connection between mitochondrial structure and epigenetic regulation is a poorly understood mechanism. As mitochondrial dynamics and function are pivotal regulators of epigenetic change that affects chromatin organization, transcription, and gene expression, we investigated the hypothesis that arsenic promotes dysfunctional epigenetic regulation of MuSC renewal and fate through critical remodeling of mitochondrial architecture. In C2C12 cells, a well-established MuSC model, we found that a single 20 nM arsenite exposure perturbs expression, post-transcriptional processing and oligomeric state of key proteins that regulate mitochondrial shape and metabolism. This strongly affected the capacity of quiescent reserve cells to transition into an advanced, committed myogenic state. Assessment of mitochondrial function revealed that mitochondrial mass, membrane potential, bioenergetics and redox state were all enhanced by arsenic, relative to control cells. Transmission electron microscopic studies suggested these alterations are accompanied by remodeling of inner mitochondrial membrane cristae and induction of autophagy. The arsenic-promoted mitochondrial phenotype regulated expression of quiescence and cycling markers and intervention with agents that scavenge mitochondrial oxidants maintained normal MuSC function. As in MuSC isolated from arsenic-exposed mice, we found that arsenic-primed reserve cells maintained their dysfunctional mitochondrial and myogenic phenotype through multiple generations in the absence of arsenic. This suggests a prolonged change in epigenetic regulation. We conclude that mitochondrial topology is not just an indicator of the differentiation state of a stem cell, but may also epigenetically govern self-renewal and fate. Our findings reveal a link between mitochondrial architecture and the regulation of the exit of a stem cell from quiescence into an activated state. Supported by NIEHS grant R01ES023696.

1251 Involvement of CNC-bZIP Protein NRF1 in Arsenic-Induced Impairments in Adipogenesis and Adipose Function

Y. Hou[1], P. Xue[2], R. Zhao[1], J. Dong[5], Z. Liu[1], J. Fu[1], H. Wang[1], Y. Xu[1], M. Yamamoto[4], Q. Zhang[3], M. E. Andersen[3], W. Qu[2] and J. Pi[1]. *[1]China Medical University, Shenyang, China; [2]Fudan University, Shanghai, China; [3]The Hamner Institutes for Health Sciences, Research Triangle Park, NC; [4]Tohoku University, Sendai, Japan and [5]Wuhan University of Science and Technology, Wuhan, China.*

Environmental arsenic exposure is a worldwide public health problem. Chronic exposure to high levels of inorganic arsenic (iAs) is associated with many human ailments including cancer, skin disorders, vascular diseases and type 2 diabetes (T2D). White adipose tissue (WAT) is an active organ that stores and releases energy, maintains glucose homeostasis, and secretes a variety of factors that influence appetite, insulin sensitivity, and inflammation. Disturbance of adipogenesis and WAT function is associated with insulin resistance, which plays an early pathogenic role in the development of T2D. iAs and its trivalent methylated metabolites inhibit adipogenesis and impair WAT function. However, the underlying mechanism remains elusive. In the present study we found that iAs, during the early stage of adipogenesis, significantly induces the protein expression of the long isoforms of NRF1 (Nuclear factor E2-related factor 1, L-NRF1), a primary transcription factor regulating adaptive antioxidant response and proteasome homeostasis. Moreover, L-NRF1 negatively regulates the expression of PPARγ, a regulator of terminal adipogenic differentiation and adipose function. In contrast, short isoforms of NRF1 (S-NRF1) derived from alternative splicing and/or post-translational modification play fundamental roles in promoting adipogenesis. Phenotypic analysis of fat-specific Nrf1-knockout mice showed that S-NRF1 are essential in the development of WAT. It appears that iAs-induced L-NRF1 disturbs the normal function of S-NRF1 leading to the effects of arsenic on adipogenesis and insulin sensitivity. Our studies highlight the importance of NRF1 in WAT and provide insight into the effects of iAs exposure on WAT development and function and on the mechanism(s) of iAs-induced T2D.

1252 Arsenic Toxicity Is Influenced by the Host-Associated Microbiome

M. P. Coryell[2], S. Schneider[2], T. R. McDermott[2] and S. T. Walk[2]. *[1]Land Resources and Environmental Science, Montana State University, Bozeman, MT and [2]Microbiology and Immunology, Montana State University, Bozeman, MT*. Sponsor: D. Keil.

There is a high degree of unexplained inter-individual variability in pathologies related to chronic arsenic exposure, even when controlling for known influences such as host genetics and exposure levels. Microorganisms, including those found in the human gut microbiome, are known to encode for arsenic-active enzymes. Previous studies have demonstrated that perturbations of the murine microbiome during arsenic exposure alter the types of excreted arsenicals in urine. However, no study has directly addressed whether the microbiome plays a beneficial or detrimental *in vivo* role in arsenic metabolism. We recently investigated the hypothesis that the mammalian gut microbiome detoxifies ingested arsenic. Mice with an intact microbiome were compared to antibiotic-treated and axenic (germ-free) mice during exposure to varying levels of inorganic arsenate. Wild-type C57BL/6 mice were considered as well as an isogenic mutant strain lacking the ability to produce the primary mammalian arsenic detoxification enzyme, arsenite methyltransferase (As3mt). Fecal pellets were collected throughout exposures for microbiome profiling via 16S-based metagenomics and for total arsenic analysis. Similarly, a variety of tissue samples were collected at the end of each experiment for arsenic analysis. Regardless of mouse genotype, higher total arsenic levels were measured in the feces of mice with an intact microbiome compared to antibiotic-treated and germ-free mice. Conversely, higher levels of arsenic were measured in tissues of antibiotic-treated and germ-free mice compared to mice with an intact microbiome. Importantly, significant dose-dependent mortality was observed in antibiotic-treated and germ-free As3mt knockout mice, while the same mice with an intact microbiome appeared relatively unaffected. Our results provide strong evidence that the gut microbiome not only alters the metabolism of ingested arsenic, but it is absolutely required for surviving arsenic exposure in the absence of host detoxification.

1253 Effects of Low-dose Arsenic Exposure on Innate Immune Signaling in Human Primary Bronchial Epithelial Cells

B. C. Goodale and B. A. Stanton. *Dept of Microbiology and Immunology, Geisel School of Medicine at Darmouth, Hanover, NH.*

Arsenic exposure is a global health concern because of its multiple adverse health effects, including increased morbidity and mortality from infectious lung disease. In the United States, *in utero* exposure to arsenic is associated with increased risk of respiratory infection. However, the mechanism by which arsenic exposure at levels relevant to the US population affects respiratory immune function is unknown. Groundwater contains primarily inorganic arsenic (iAs), which is metabolized *in vivo* to organic arsenic species including monomethlyarsinous acid (MMA), and dimethylarsinic acid (DMA). Foods such as rice and fruit juices are additional sources of arsenic exposure, and can contain both iAs and organic arsenic species. We exposed differentiated primary human bronchial epithelial cells (HBECs) to increasing concentrations of an arsenic mixture representative of levels detected in human serum (50% DMA, 25 % MMA and 25% iAs). Following 6 days of exposure to the arsenic mixture, we challenged the airway cells with Pseudomonas aeruginosa (Pa), a gram-negative opportunistic bacterial pathogen, and examined cytotoxicity, cytokine release and RNA expression in the HBECs. Neither exposure to the arsenic mixture (0-50 ppb total arsenic) nor Pa increased cytotoxicity as detected by lactate dehydrogenase activity. Cells from both male and female donors exhibited variability in basal expression and maximal induction of cytokines, but Pa induced robust release of all cytokines in all donors tested (N=6). The arsenic mixture alone significantly increased IL-8 and CXCL1 release in a concentration-dependent manner, suggesting an inflammatory phenotype, while arsenic effects on Pa-induced cytokine release were variable between donors. Further studies are focused on identifying genes modulated by arsenic and Pa that lead to differential cytokine secretion in primary HBECs, and may underlie adverse effects of arsenic on respiratory function in human populations.

1254 Dose-Response of Low-Dose Arsenite Cytotoxicity in a Human Keratinocyte Cell Line

K. Pennington[2], K. T. Bogen[1], L. Arnold[2], A. Chowdhury[2] and S. M. Cohen[2]. *[1]Exponent Health Sciences, Oakland, CA and [2]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE.*

The *in vitro* arsenite (AsIII) cytotoxicity dose-response (DR) of a human keratinocyte cell line (HEK001) was examined in detail using the MTT assay to determine cell survivability. Fifty-four 96-well plates were treated with AsIII concentrations of 0.25, 0.5, 1, 2, 3, 4, 5, 7, 10, 15, 20, 25, or 30 µM. Plate-specific relative survival (%) was estimated as 100% times the ratio of measured viability in exposed to unexposed cells. Because of unexpected variation in survival response patterns, a two-stage DR analysis was used, in which the plate-specific survival data were initially fit to a generalized lognormal response function positing proportions P and Po of relatively highly sensitive and resistant cells, respectively. To examine the DR pattern for the remaining (1-P-Po) fraction of cells that appeared to exhibit typical levels of AsIII sensitivity, plate-specific P and Po estimates were used to adjust data from all 54 plates (as well as from a subset of 28 plates that exhibited the best initial fits) to reflect the condition that P = Po = 0. Although four different DR models, fit to the combined adjusted data averaged at each AsIII concentration, were each very predictive (R2 > 0.97), none were statistically consistent with each data set examined (p < 10-5). The adjusted mean responses at ≤ 3µM were approximately equal (p > 0.30) and exceeded 100% significance (p ≤ 10-6). A model with low-dose hormetic behavior provided the best fit to the combined data (R2 = 0.995). Markedly variable DR patterns were observed in this HEK001cell survival study that are difficult to interpret with precision, and merit comparison with results using alternative assays of cell survival.

1255 Genomic Targets of Sex Steroid Hormones for the Arsenic Metabolism Regulation in HepG2 Cells

C. O. Puente-Valenzuela, J. J. Duarte-Sustaita, A. Mendez-Hernandez and G. G. García-Vargas. *Universidad Juarez del Estado de Durango, Gomez Palacio, Mexico.* Sponsor: L. M. Del Razo.

Epidemiologic studies suggest that women are more efficient to excrete arsenic and its metabolites, suggesting hormonal regulation. The aim of this work was to characterize the arsenic species concentrations, and the effect of testosterone, progesterone and estradiol on the gene expression of AS3MT, CBS, BHMT, GSR and GCLC in the human hepatocytes cell line HepG2. The gene expression was evaluated using TaqMan® assays at StepOne Real-Time PCR system of Applied Biosystem; arsenic species were quantified by the method of cryo-trapping (HG-CT-AAS; in an AAnalyst 800 of Perkin Elmer). The cells were individually stimulated with testosterone (0, 3.12, 6.25 and 12.5 µg/mL), progesterone (0,15.0, 30.0 and 300 µg/mL) and estradiol (0, 0.5, 1.0 and 6.0 µg/mL) at 0, 6, 18 and 24 h and exposed to 0.2 µM of arsenite (Sodium Meta-Arsenite) at 6 h of started the experiment, each experiment had 30 h of duration. Treatments of 1.0 and 6.0 µg/mL of estradiol and 30.0 µg/mL of progesterone had 2-4 fold more gene expression of BHMT and CBS with respect of testosterone and basal treatments and almost 2 fold more in the gene expression of GSR; with respect of gene expression of AS3MT only the treatment of 15.0 µg/mL was 3 fold higher that testosterone and basal treatments. On the arsenic metabolism there were not differences between treatments, the most relevant difference was in the efflux of DMA, where the treatments of 1.0 and 6.0 µg/mL of estradiol and 15 and 30 µg/mL of progesterone had an efflux 2 fold more higher that testosterone treatments, also they had less total arsenic in the intracellular compartment. Finally, the overexpression of genes BHMT, CBS and AS3MT by female steroid hormones did not change the profile of arsenic species. In this model, the main change observed was in the efflux of DMA and the arsenic intracellular accumulation; this can explain the differences found in the epidemiological studies.

1256 Arsenic Transformation of Human Prostate Epithelial and Stem Cells Show Decreased Toll Like Receptor -3 (tlr3) Expression: Correlation with Down-Regulated miR 29, 147 And 152 Clusters and Increased Expression of DNA Methyl Transferases Dnmt1, 3a and 3b

C. Escudero-Lourdes[1], E. E. Uresti-Rivera[2], N. O. Ngalame[2], M. P. Waalkes[2] and E. J. Tokar[2]. [1]*Inmunotoxicologia, Universidad Autonoma de San Luis Potosi, San Luis Potosi, Mexico and* [2]*National Toxicology Program, National Institute of Environmental Health Sciences, Durham, NC.*

Toll-like receptors (TLRs) are a family of transmembrane receptors that recognize pathogen-associated molecular patterns. TLR-3 is activated by double-stranded RNA (dsRNA) from viral genome, intermediates formed during viral replication cycle, or from damaged cells. TLR-3 can also be activated by miRNAs secreted by neighbor cells. TLR-3 is known to play an important role in tissue regeneration and wound healing but its role in cancer is controversial since it can play a pro- or anticancer role depending on the cell type. TLR3 activation by the agonist poly polyinosinic:polycytidylic acid (Poly I:C), induces miRNA-29b, -29c, -148b, and -152 upregulation in prostate tumor-derived cell lines and primary tumors. These microRNAs induced re-expression of epigenetically silenced oncosuppressor retinoic acid receptor beta (RARβ) by targeting DNA methyltransferases, rendering cells sensitive to retinoic acid-induced apoptosis both *in vitro* and *in vivo*. Exposure to inorganic arsenic (iAs) is associated with prostate cancer in exposed human populations. *In vitro*, chronic iAs exposure malignantly transforms a human prostate cell line RWPE-1 (29 weeks to form CAsE-PE cell line) and its isogenic stem cell line WPE-stem (only 18 weeks to form As-CSC cells). Since both iAs-transformed complete and stem cells show anchorage-independent growth and form tumors in mice, in the current study, we hypothesized that the TLR-3-associated anti-cancer pathway is impaired in these cells, making the cells unresponsive to TLR-3 activation. As expected, CAsE-PE and As-CSC showed a significant downregulation of TLR-3 and decreased expression of miR29c*_1, 148-b, 152-1, 152_5p-1. DNMT1, DNMT3a, and DNMT3b, which are normally targeted by these miRNAs, were overexpressed in both CAsE-PE cells and AsCSC cells. RARβ protein showed no changes in CAsE-PE or As-CSC cells. These results show that the TLR-3 anti-tumor pathway which induces the re-expression of the oncosupressor RARβ is impaired in iAs-transformed human prostate cells.

1257 Specificity Protein (Sp) Transcription Factors Are Downregulated in Arsenite - Transformed Human Urothelial Cells (UROtsa)

V. Nair, S. Somji, S. H. Garrett and D. A. Sens. *Pathology, University of North Dakota, Grand Forks, ND.*

Cell transformation by heavy metals are generally considered to take place through the activation or repression of several target genes. Previous reports have characterized the downregulation of SPARC (Secreted Protein, Acidic and Rich in Cysteine) gene in human urothelial cells (UROtsa) transformed by heavy metals. SPARC is a matrix cellular protein that mediates cell matrix interactions. The transcriptional regulation of the SPARC gene expression has not been fully understood. Specificity protein (Sp) transcription factors are zinc finger transcription factors which are involved in the constitutive expression of the SPARC gene. In this study we evaluated the effect of transformation of UROtsa cells with arsenite on Sp transcription factor. Transformation with arsenite resulted in the downregulation of expression of Sp1 and Sp3 transcription factors and caused repression of SPARC mRNA in the malignantly transformed urothelial UROtsa cells. Treatment of parental UROtsa cells with 1, 3 or 6 µM arsenite also downregulated the expression of SPARC and Sp proteins. The role of Sp transcription factor in regulating SPARC expression was investigated by knockdown of Sp1 (iSp1), Sp3 (iSp3), or their combination (iSp1/3) by RNA interference in the parental UROtsa cells and this resulted in decreased expression of SPARC. In addition our studies have shown that downregulation of SPARC protein by arsenite is attenuated by co-treatment with the antioxidant - glutathione (GSH) indicating the role of ROS (Reactive Oxygen Species) in arsenite dependent downregulation of SPARC. The underlying mechanism of action of heavy metals like arsenite and cadmium on repression of SPARC and down regulation of Sp1 and Sp3 transcription factors in UROtsa cells are currently being investigated.

1258 A Role for Anterior Gradient 2 in a MCF-10A Cell Model of Arsenic Induced Breast Cancers

J. L. Van Gieson, S. H. Garrett, D. A. Sens and S. Somji. *Pathology, University of North Dakota, Grand Forks, ND.*

Arsenic and cadmium are environmental toxicants that have been implicated in the development of various cancers including breast cancer. Previous studies from our laboratory have shown that both arsenite and cadmium can malignantly transform the breast epithelial cell line, MCF-10A. Global gene expression analysis of the transformed cells showed the over-expression of proto-oncogene Anterior Gradient 2 (AGR2) in the arsenite transformed cell line when compared to the parent and the cadmium transformed cells. Recent studies have shown that AGR2 plays a prominent role in mediating pro-oncogenic signals of ER, and there is a correlation between increased AGR2 expression and poor outcome of therapy in patients with ERα-positive breast cancers. The goal of this study was to determine the role of AGR2 in the development of arsenite and cadmium induced breast cancers. Our study shows that the expression of AGR2 is significantly increased in MCF-10A cells transformed with arsenite when compared to cadmium transformed cells. Exposure of the parent MCF-10A cells to arsenite for 48 hours resulted in a significant increase in AGR2 expression suggesting that arsenite has the potential to induce AGR2 in MCF-10A cells. In order to further investigate the effects of AGR2 expression on breast epithelial cells, the parent MCF-10A cells and cadmium transformed MCF-10A cells were stably transfected with the AGR2 gene. Over-expression of AGR2 in the MCF-10A cells increased the ability of the cells to migrate faster in the wound scratch assay. However transfection of the cadmium transformed MCF-10 cells with AGR2 resulted in slowing the ability of the cells to migrate in the assay. Thus, it seems that the effect of AGR2 expression on cell migration is different in the transformed versus the non-transformed cells. Furthermore, our data also shows that the expression of AGR2 in the MCF-10A cells is not regulated by the estrogen receptor status of the cells but may be regulated by epigenetic mechanisms.

1259 Urinary Metabolomic Shifts in Pregnant Women Exposed to Arsenic

K. Bailey[2], E. Martin[5], J. Laine[5], A. Olshan[5], L. Smeester[5], Z. Drobna[5], M. Styblo[5], M. Rubio-Andrade[1], G. Garcia-Vargas[1] and R. Fry[5]. *[1]Facultad de Medicina, Gomez Palacio, Mexico; [2]Syngenta Crop Protection, LLC, Research Triangle Park, NC; [3]Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC; [4]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC and [5]Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Millions of people worldwide are exposed to levels of inorganic arsenic (iAs) in their drinking water that are associated with detrimental health effects. iAs exposure during pregnancy is of particular concern as it has been associated with adverse pregnancy and birth outcomes. Children exposed to iAs *in utero* also have increased susceptibility to numerous adverse health effects throughout their lifetimes, including the development of non-communicable diseases in adulthood. Urinary metabolic profiling is an attractive method for monitoring iAs-exposed populations as it is non-invasive and has the potential to identify biomarkers of exposure, toxicity, and disease susceptibility. We performed urinary metabolomic profiling on 50 pregnant women from a prospective pregnancy cohort from Gómez Palacio, Mexico. These women were exposed to varying levels of iAs in their drinking water, ranging from below the limit of detection to 236 µg/L. Levels of total urinary arsenic (U-tAs) ranged from 6.19 to 319.7 µg/L. A total of 417 urinary metabolites were identified, of which 27 had a significant association between levels in urine and U-tAs. These metabolites were enriched in functions including amino acid metabolism and nucleic acid metabolism, and several had known associations with inflammation, oxidative stress, and iAs-associated disease. Future work will determine if these urinary metabolite profiles can be used to identify iAs-exposed populations and predict adverse health outcomes.

1260 Tobacco Smoking Decreases Arsenic Methylation Capacity in Alcohol Abstemious Women in Northern Mexico

L. Lopez-Carrillo[1], E. E. Félix-Arellano[1], C. Hernández-Alcaraz[1], R. U. Hernández-Ramírez[1], A. J. Gandolfi[2] and M. E. Cebrian[3]. *[1]Centro de Investigación en Salud Pública, Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico; [2]College of Pharmacy, University of Arizona, Tucson, AZ and [3]Toxicology, Cinvestav-IPN, Mexico City, Mexico.*

Some, but not all published studies have suggested that smoking interferes with arsenic (As) methylation. However, there is no general agreement as to the methylation indexes affected and its potential impact on As-related pathology. We evaluated the association between tobacco smoking and As methylation capacity in women moderately exposed to As. A cross-sectional study was performed in 910 (677 non-smokers and 233 smokers) clinically healthy women exposed to As via water consumption in Northern Mexico, and that reported no alcohol consumption, a factor potentially interfering with As methylation. The profile of As metabolites in urine was determined by HPLC/ICP-MS. Methylation capacity was assessed by calculating the percentage of As species and the primary [MMA(V)/(As(III)+As(V)] (PMI) and secondary [DMA(V)/MMA(V)] methylation (SMI) indexes. Lifetime pack years were calculated according to the NCI index: one lifetime-pack year is equivalent to consume one pack of cigarettes per day during one year. The relationship between As methylation capacity and lifetime pack-years of tobacco smoking was assessed by multiple linear regression models. The median urinary TAs value was 26 µg/gcreat (1.3 - 7,047.3). About 10% of women had values above 150 µg/gcreat. Tobacco smokers had marginally higher MMA and DMA levels. %MMA was significantly higher and %DMA marginally lower. PMI values were marginally higher (p=0.08) in smokers whereas SMI values were significantly lower. Our results were in good agreement with previous findings in other populations with diverse ethnic origin. Further studies are needed to elucidate the contribution of smoking and impaired As methylation to the risk of As-related pathology. Supported by CONACYT, Mexico (FOSSIS: 2005-C02-14373, 2009-01-11384 and 2010-1-140962) and by The Dean Carter Binational Center for Environmental Health Sciences and the NIEHS Superfund Research Program (ES 04940).

1261 Association Between Variants in Arsenic Methyltransferase (*AS3MT*) and Urinary Metabolites of Inorganic Arsenic: Role of Exposure Level

X. Xu[7], Z. Drobna[7], S. Voruganti[7], K. Barron[7], C. González-Horta[4], B. Sánchez-Ramírez[4], L. Ballinas-Casarrubias[4], R. Hernández Cerón[2], D. Viniegra Morales[2], F. Baeza Terrazas[2], M. Ishida[4], D. Gutiérrez-Torres[4], J. Saunders[7], R. Fry[7], D. Loomis[3] , G. García-Vargas[5], L. M. Del Razo[1], M. Styblo[7] and M. Mendez[7]. *[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México DF, Mexico; [2]Colegio de Médicos Cirujanos y Homeópatas del Estado de Chihuahua, Chihuahua, Mexico; [3]International Agency for Research of Cancer, Lyon, France; [4]UACH, Chihuahua, Mexico; [5]Universidad Juárez del Estado de Durango, Durango, Mexico; [6]Environmental Sciences and Engineering, UNC Chapel Hill, Chapel Hill, NC and [7]Nutrition, UNC Chapel Hill, Chapel Hill, NC.*

Polymorphism in AS3MT has been shown to influence arsenic (As) metabolism. It is not known, however, whether the influence of AS3MT polymorphisms on As metabolism may vary at different levels of As exposure. We investigated, in a population of Mexican adults exposed to inorganic As in drinking water, whether associations between seven AS3MT variants and urinary As metabolites were consistent with prior studies, and whether these associations varied depending on the level of exposure. Overall, associations between As metabolites and AS3MT variants were consistent with the literature. Referent alleles (those previously associated with a higher percentage of dimethyl-As (DMAs%) in urine) were associated with significantly higher DMAs% and ratio of DMAs to methyl-As (MAs), and significant reductions in MAs% and inorganic As%. For four variants, associations with measures of As metabolism were significantly stronger among subjects exposed to drinking water As >50 ppb vs. ≤50 ppb (water X variant interaction P<0.10). For example, for rs10748835, the mean (SE) reduction in DMAs% associated with having non-referent alleles was -3.7 (SE) vs. -1.3 (SE) among participants with higher vs. lower water As. In contrast, for one variant (rs1788125), associations were significantly stronger at exposures ≤50 ppb. Results suggest that As exposure may influence the extent to which several AS3MT variants affect As metabolism. The variants most strongly associated with As metabolism–and perhaps with susceptibility to As-associated disease–may vary in settings with lower vs. higher exposure.

1262 DNA Methylation Characteristics of Arsenic-Associated Diabetes in a Prospective Cohort in Chihuahua, Mexico

E. Martin[5], K. Bailey[5], L. Smeester[5], C. González-Horta[3], B. Sánchez-Ramírez[3], L. Ballinas-Casarrubias[3], M. Ishida[3], D. Gutiérrez-Torres[3], R. Hernández Cerón[2], D. Viniegra Morales[2], F. Baeza Terrazas[2], L. M. Del Razo[1], G. García-Vargas[4], J. Buse[5], D. Loomis[6] , Z. Drobna[5], M. Styblo[5] and R. C. Fry[5]. *[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Chihuahau, Mexico; [2]Colegio de Médicos Cirujanos y Homeópatas del Estado de Chihuahua, Chihuahua, Mexico; [3]Universidad Autónoma de Chihuahua, Chihuahua, Mexico; [4]Universidad Juárez del Estado de Durango, Gómez Palacio, Mexico; [5]Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC and [6]Monographs Section, IARC, Lyon Cedex, France; [7]School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

The Chihuahua Cohort was established to study the effects of inorganic arsenic (iAs) induced chronic diseases, specifically diabetes mellitus (DM). In the present study, we profiled CpG methylation differences in diabetic (n=91) and non-diabetic (n=86) individuals of the cohort in association with arsenic exposure to identify a methylation "finger print" of arsenic-associated diabetes. Methylation data were analyzed both in an untargeted manner and looking specifically at genes previously associated with arsenic exposure as well as those previously associated with diabetes mellitus. Methylated Island Recovery Assay (MIRA) was used to determine methylation levels of individuals in this study. Urinary Total Arsenic (U-tAs) (range 4.69-445.2 µg/L) was used to determine individuals' exposure to arsenic, and levels of fasting plasma glucose (FPG) and 2 hour blood glucose (2HBG) were used to determine an individual's diabetes status. We identified a total of 354 genes associated with arsenic exposure in diabetic individuals and, a total of 3273 genes associated with arsenic exposure in non-diabetic individuals. These data are similar to data from our previous metabolomics assessment where diabetic individuals were found to have more altered metabolites in association with arsenic exposure when compared to non-diabetic individuals.

1263 DNA Methylation Change of LINE-1 Associated with Chronic Arsenic Exposure Through a Cross Sectional Study in Bangladesh

T. Suzuki[1], K. Hossain[2], S. Himeno[3] and K. Nohara[1]. *[1]Center for Environmental Health Sciences, National Institute for Environmental Studies, Tsukuba, Japan; [2]Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi, Bangladesh and [3]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.*

Naturally occurring inorganic arsenic is known to cause serious health problems. Recently, it has been reported that epigenetic modifications including the changes of DNA methylation are associated with several diseases. However, limited information is available on the association between chronic arsenic exposure and DNA methylation levels in human. In this study, we explored the relationship between chronic arsenic exposure and DNA methylation levels of LINE-1 in blood genomic DNA through a cross-sectional study. A total of 239 human subjects, 176 from arsenic-endemic and 63 from non-endemic areas in rural Bangladesh, were recruited for this study. Arsenic concentrations in the drinking water and nails of the study subjects were measured by ICP-MS, and LINE-1 methylation levels were measured by bisulfite pyrosequencing. We newly indicated that LINE-1 methylation levels were significantly lower in arsenic-endemic population than those in non-endemic population. There were also significant negative correlations of drinking water and nail arsenic concentrations with LINE-1 methylation levels. Inverse relationships of arsenic exposure with methylation levels were also significant even after adjusting for relevant covariates including age, sex, BMI and smoking. Furthermore in linear regression analysis, our results showed that sex was a significant confounder in the arsenic-related LINE-1 methylation, and subsequent analysis indicated that drinking water and nail arsenic concentrations were significantly correlated with LINE-1 methylation in females but not in males. These results indicated that chronic arsenic exposure was inversely associated with LINE-1 methylation, and females were more susceptible to arsenic-related LINE-1 methylation than males.

1264 Risks Associated with Arsenic Exposure Resulting from the Consumption of California Wines Sold in the United States

A. Monnot[3], B. Tvermoes[2], R. Gerads[1], H. Gurleyuk[1] and D. Paustenbach[3]. *[1]Brooks Rand Labs, Seattle, WA; [2]Cardno-ChemRisk, Boulder, CO and [3]Cardno-ChemRisk, San Francisco, CA.*

Concerns have recently been raised about the presence of arsenic (As) in wine and the potential effects on human health. In order to characterize the difference between red, blush and white wines, 81 different California wines (99 total boxes or bottles of wine) were evaluated for organic and inorganic As concentration. The average concentrations of total inorganic As in red, blush and white wines were 6.4 µg/L (range: 0.3-20.6 µg/L), 27.6 µg/L (range: 14.7-41.2 µg/L) and 9.7 µg/L (0.6-30.4 µg/L), respectively. The average concentrations of total organic As in red, blush and white wines were 0.6 µg/L (range: 0.1-2.7 µg/L), 1.15 µg/L (range: 0.7-2.2 µg/L), and 0.45 µg/L (range: 0.1-1.7 µg/L), respectively. A screening level risk assessment was conducted to assess the potential non-carcinogenic risk resulting from the consumption of these wines. The hazard quotient (HQ) for the inorganic As based on the current EPA RfD and the As content of red, blush and white wines was each less than one; indicating that the non-cancer health risk was insignificant. The epidemiologic data indicate there is not an increased cancer risk via oral ingestion of inorganic arsenic at doses less than approximately 3 µg/kg-day (JEFCA 2011) which is more than that associated with ingesting three glasses per day of wine (0.22 µg/kg-day using the 95% UCL). This analysis indicates that, based on the available data, ingestion of California wines at reasonable doses does not pose a hazard due to inorganic As content.

1265 Rice, Arsenic, and Public Health: Consumption of Rice Products Containing Low Levels of Arsenic and Its Relationship to Health

L. A. Fahnestock, J. Sabate, R. D. Smith, M. Randhawa, G. Siapco and S. Soret. *Public Health, Loma Linda University, Loma Linda, CA.*

Over the last few years, individuals have become increasingly concerned regarding safety of food products. Even more currently, literature discussing the issue of arsenic "tainted" grain products has spurred concern among the general public. Researchers have maintained that high levels of arsenic in drinking water have been correlated to negative health outcomes; but the dietary exposures from food items containing levels well below the directed water standards, have become a subject of increasing evaluation and priority. The possible health ramifications of consuming a diet rich in rice, specifically regarding low-level arsenic exposure through rice ingestion, is not completely understood. Considering the implication that low levels of arsenic may be directly linked to specific cancers and that the majority of rice and rice products have been shown to contain measurable levels of arsenic, the purpose of this study is to explore the association between overall rice consumption of adults within the United States (18 years and older) with total urinary arsenic levels and arsenic specific disease outcomes. In the National Health and Nutrition Examination Survey, over 5,000 individuals per year were sampled via a complex probability analysis between years 2003-2012. From the surveys and laboratory samples, the data analysis will be run utilizing logistic regression models in order to test the associations between rice consumption, urinary arsenic levels, and health outcomes (high blood pressure, high blood glucose, and bladder and skin cancer). Early stage evaluations support the research hypothesis, which indicates that arsenic containing rice products can possibly contribute to various disease states. Trying to convey at first look, association appears likely; more detailed analysis will show if that is true, and to what degree of association is found in regards to specific diseases.

1266 Antioxidants Ameliorate Impairment of Vascular Smooth Muscle Cell Mitochondrial Function by Monomethylarsonous Acid

J. Angermann[2], C. Pace[2], T. Das Banerjee[2] and R. Dagda[2]. *[1]Department of Pharmacology, University of Nevada, Reno, NV and [2]School of Community Health Sciences, University of Nevada, Reno, NV.* Sponsor: J. Angermann.

Arsenic exposure is associated with vascular disease, yet cellular mechanisms of toxicity are largely unknown. The present study investigates mitochondrial dysfunction in relation to the vascular toxicity of arsenic. The relative effects of inorganic arsenic (iAs) and the metabolite monomethylarsonous acid (MMA) on mitochondrial function in aortic vascular smooth muscle cells (VSMC) were assessed through cell proliferation, cell death, oxygen consumption rates, glycolysis, mitochondrial morphology, ATP generation, and expression of several mitochondrial proteins. Survival curves and determination of mitochondrial function following a 24-hr. exposure indicate that MMA is significantly more toxic than iAs. A 3 hr. exposure of MMA, but not iAs, caused a significant decrease in basal and maximal respiration and a concomitant increase in compensatory glycolysis as measured by an XF24 Extracellular Flux Analyzer. Steady state levels of mitochondrial ATP were not impacted by either arsenic species at 3 hrs. indicating that the loss of oxygen consumption is upstream of ATP loss. MMA but not iAs treatment caused a significant increase in hydrogen peroxide levels. Exposure of VSMCs to high doses of MMA fragmented mitochondria and caused a decrease in the percent of mitochondria occupied by cytosol. In contrast, treating VSMCs with a high dose of MMA for 6 hrs. or a low dose for 24 hrs. enhanced mitochondrial activity as assessed by single cell analysis of mitochondrial content or by TOM20 immunoblotting. Catalase and MnTBAP pretreatment of VSMCs was protective against multiple facets of mitochondrial dysfunction, but MnTBAP was unable to reverse compensatory glycolysis. Overall our data demonstrate that MMA, but not iAs, is a mitochondrial-specific intoxicant in VSMCs and that MMA-induced structural changes in mitochondria and glycolytic shifts are likely compensatory manifestations secondary to mitochondrial dysfunction and ROS elevation.

1267 Hyper-Resistance to Acute Arsenic Toxicity of CD34+ Epidermal Cells Enriched from HaCat Keratinocytes

X. Wu, J. Fu, H. Wang, Y. Hou, J. Pi and Y. Xu. *China Medical University, Shenyang, China.*

Arsenic is indentified as a human carcinogen with the skin a well-known target. Accumulation of cancer stem cells during the process of arsenic carcinogenesis is observed in previous *in vitro* and *in vivo* studies, including in arsenic-transformed HaCaT human keratinocytes. To elucidate underlying mechanisms, CD34+ cells from HaCaT keratinocytes were isolated with immunomagnetic separation method. Stem cell-like characteristics and resistance to acute cytotoxicity of sodium arsenite (NaAsO2) of CD34+ cells were tested. The enrichment rate of CD34+ cells by immunomagnetic separation was (88.6 ± 2.5)% (compared with pre-separation, $p < 0.001$) as determined by flow cytometry. Spheroids derived from single CD34+ cells could be formed and serial passaged in Matrigel, suggesting a capacity of self renewal. Holoclone forming capacity of CD34+ cells was 2.4 fold of control ($p < 0.01$). Transcript levels of CD34, p63, K5, K14, OCT4 and SHH, all stem cell markers, in CD34+ cells were significantly increased ($p < 0.05$), which were 18.2

fold, 2.2 fold, 1.6 fold, 3.9 fold, 2.3 fold and 4.2 fold of control, respectively. Viability of CD34+ cells after 24-hour exposure to NaAsO2 (20 to 80 µmol/L) was significantly higher than that of control ($p < 0.05$). Lethal concentration 50 (LC50) of CD34+ and control cells in response to NaAsO2 was 98.3 µmol/L and 50.1 µmol /L, respectively. Transcript levels of genes encoding drug transporter (ABCC1 and ABCG2), detoxification enzyme (GST-Pi) and anti-oxidative enzymes/proteins (HO-1 and GCLC) were significantly higher in CD34+ cells compared with control ($p < 0.05$). In conclusion, CD34+ cells from HaCaT cells show stem-cell-like phenotypes and hyper-resistance to acute arsenic toxicity. This hyper-resistance to arsenic may be related to the high expression of drug transporter genes, detoxification enzyme genes and antioxidant genes in CD34+ putative epidermal stem cells, and furthermore contributes to accumulation of stem cells during arsenic exposure.

PS 1268 Exposure to Endocrine Disrupting Heavy Metals During Pregnancy: Infant-Related and Mother-Related Outcomes

J. Gomes[2], A. Rahman[2] and P. Kumarathasan[1]. [1]*Environmental Health Sciences and Research Bureau, Health Canada, Ottawa, ON, Canada and* [2]*Health Sciences, University of Ottawa, Ottawa, ON, Canada.* Sponsor: J. Gomes.

Objectives: Lead, arsenic, cadmium, and mercury are hormonally active heavy metals and exhibit endocrine disrupting properties. Because environmental exposures to endocrine disrupting chemicals are omnipresent, it is imperative to identify adverse health outcomes that are related to these exposures. The literature appears to indicate gaps in research regarding the reproductive health effects of exposure to endocrine disrupting heavy metals. This systematic review attempts to summarize the evidence relating the potential association between exposure to toxic heavy metals and adverse health outcomes for the mother and infant during pregnancy. Methods: Five databases were searched for original research and review articles. After all screening processes, 48 articles were retained for data extraction. The methodological quality of observational studies was assessed and scored using a modified version of the Downs and Black checklist. The AMSTAR checklist was used for assessing review studies. Results: Lead, arsenic, and cadmium exposure were associated with fetal loss outcomes such as spontaneous abortion, stillbirth, and neonatal death. With respect to mother-related outcomes, studies demonstrated significant associations between low levels of lead exposure and pregnancy hypertension. An association between arsenic exposure and gestational diabetes was also observed. Conclusion: Heavy metals appear to be associated with impaired fetal growth, gestational diabetes, spontaneous abortions and stillbirth. Although some studies suffer from methodological issues including problems with exposure assessment; the findings are consistently indicative of adverse health outcomes for the mother and the infant.

PS 1269 Assessing Human Metal Accumulations in an Urban Superfund Site

K. Hailer[1], M. Calhoun[1], B. West[1] and L. Lin[2]. [1]*Chemistry, Montana Tech, Butte, MT and* [2]*Statistics, Montana State University, Bozeman, MT.*

Butte, Montana, a town of approximately 35,000 residents, is part of the largest Superfund site in the continental United States. However, open-pit copper and molybdenum mining continues in close proximity to Butte's urban population. Even with current surface mining, county air sampling has focused on PM 2.5 micron particulate with little focus on any larger particulate circulation. A preliminary study conducted in 2013 found statistically higher levels of copper, manganese, and molybdenum in the hair of residents living in Butte as compared to a control population in Bozeman, MT. In addition, microRNA analysis showed several statistical differences in inflammatory and oxidative stress pathways in the Butte population. In this current study, a larger number of volunteers from Butte were recruited to submit hair and blood samples (n=105). Having no history of mining activity, Bozeman was again used as the control population (n=86). Hair samples were analyzed for 36 elements and blood samples were analyzed for 11 elements both by inductively coupled plasma-mass spectrometry. In addition, soil samples (n=32) from some Butte volunteers were collected and analyzed for 35 elements by ICP-MS. Also, three air monitors were stationed in Butte for 4 months collecting total suspended particulate matter as well as PM 10 micron particulate. Filters from the air monitors were collected weekly and analyzed for 35 elements by ICP-MS. Hair analysis revealed statistically high levels of copper, manganese, molybdenum, aluminum and arsenic in the Butte population. Only aluminum and arsenic were found to be statistically elevated in the blood samples of Butte residents. All soil samples have revealed detectable levels of arsenic, lead, and cadmium, with some samples having arsenic concentrations above allowable EPA limits. These data illustrate the need for continued study and data collection from individuals living within Butte to determine possible current or historical environmental exposure routes.

PS 1270 Perturbation of the Fecal Microbiome from Metal Exposed Rats

J. A. Lewis[2], B. C. Dancy[2], C. L. Horton[2], Y. S. Lee[1], M. S. Madejczyk[1], G. Ackermann[4], G. Humphrey[4], R. Knight[4] and J. D. Stallings[2]. [1]*Fellow, Oak Ridge Institute for Science and Education, Fort Detrick, MD;* [2]*US Army Center for Environmental Health Research, Fort Detrick, MD;* [3]*Dept of Computer Science and Engineering, University of California, San Diego, La Jolla, CA and* [4]*Dept of Pediatrics, University of California, San Diego, La Jolla, CA.*

In recent years it has become increasingly evident that the microbiome plays a critical role in human health and disease, yet there are still relatively few studies that have directly compared the effects of different toxicants on it. In this study, we compare the differential effects of several toxic metals on the fecal microbiome of exposed rats. Sprague Dawley rats were exposed to three doses of arsenic ($NaAsO_2$), chromium ($Na_2Cr_2O_7$), nickel ($NiCl_2$), cadmium ($CdCl_2$), cobalt ($CoCl_2$), or a vehicle control for one or five days via oral gavage. Fecal samples were collected prior to initial dosing and 24 hours post the final dosing. The V3/V4 region of 16S ribosomal RNA gene was amplified from DNA extracted from fecal samples, sequenced, and used to quantify microbial composition. Operational taxonomic units (OTUs) were identified that show dose dependent changes as a result of exposure to each metal. Interestingly, many of these changes are specific to an individual metal or a subset of the metals. The differential effects on the microbiome of animals exposed to different metals should be considered in future studies to assess mechanisms of toxicity and in determining risk assessment. This research was supported in part by an appointment to the Postgraduate Research Participation Program at the USACEHR administered by the ORISE through an interagency agreement between the US DOE and USAMRMC. Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.

PS 1271 Effects of Heavy Metals on Calcium Oxalate (CaOx) Crystal Formation in a *Drosophila melanogaster* Model of Nephrolithiasis

G. M. Landry[2], T. Hirata[2], C. J. Gallo[2], P. Cabrero[1], K. Strohmaier[2], P. Williams[2], J. A. Dow[1], E. Furrow[3] and M. F. Romero[2]. [1]*Biomedical and Life Sciences, University of Glasgow, Glasgow, United Kingdom;* [2]*Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN and* [3]*Veterinary Clinical Sciences, University of Minnesota, St. Paul, MN.*

Kidney stones are common, painful, and, when untreated can produce renal complications or failure. Currently, the US spends >$10.3 billion for treatment and care of individuals with kidney stones, i.e.,~1/3rd of the total NIH budget. The most common stone-type is composed of calcium oxalate (CaOx) (>80% of all stones). Interestingly, a number of investigators have reported that environmental exposure to exogenous, as well as endogenous heavy metals, particularly cadmium (Cd2+), cobalt (Co2+), and zinc (Zn2+), are associated with a greater risk of kidney stone formation. Recent genetic studies have also revealed that varied expression of the apically-localized zinc importer, Zip10 (Slc39a10), may play a significant role in CaOx nephrolithiasis. Our laboratory has developed a useful genetic model in Drosophila where major renal pathophysiology (CaOx kidney stones), particularly cation/anion movement, can be studied rather precisely. Female flies (5-10 days old) were fed sodium oxalate (NaOx, 10 mM) ± Cd2+, Co2+, or Zn2+ (100 µM) for 48 h at 28°C. Malpighian tubules (MT) were dissected and imaged using differential interference contrast (DIC) microscopy. CaOx crystals were counted, and total crystallization area, as well as average area per crystal was measured using Image J. Cd2+and Co2+ had no effect on crystal parameters in flies fed NaOx + Cd2+ or Co2+; however, in flies fed NaOx + Zn2+ with Zip10 knocked down in the MT, crystal count, total area, and average area per crystal were significantly increased. These data indicate a protective role for Zip10 to remove excess Zn2+ from the tubule lumen, and prevents Zn2+-induced detrimental effects on CaOx crystallization. As the current experimental system requires significant metal uptake by the gut, future studies will determine effects of Cd2+

and Co2+ on CaOx in microdissected MTs. We also plan to investigate the role of metal transporter expression in the development of CaOx nephrolithiasis. These combined approaches will allow us to determine if sub-toxic levels of heavy metals increase CaOx stone risk before toxic levels cause well-documented overt kidney damage.

1272 Environmental Metals and Stress Hormones: Do Lead, Cadmium, or Cortisol Promote the Shortening of Telomeres?

H. Alkhafaf, R. Lichtler, K. Esteves, S. Drury and J. Wickliffe. *Global Environmental Health Sciences, Tulane University, New Orleans, LA; Psychiatry, Tulane University, New Orleans, LA.*

Cadmium and lead are hazardous metals that are found in high levels in certain populations, with subsets that also have high stress levels. Cadmium can damage the respiratory and renal systems. Lead can also damage the renal system as well as the neurological and hematological systems. In this experiment, cortisol is used as a biological representation of high stress levels. Our hypothesis is that lead and cadmium, when combined with cortisol, may promote the shortening of telomeres and accelerate biological aging of cells and tissues. Telomere length in primary cells shortens as they are cultured *in vitro*. Telomeres of untreated, senescent cells shortened as a function of time in culture. Lead combined with cortisol resulted in a significant decrease in telomere length compared to control cells. Cadmium appeared to interfere with telomere shortening in our time-course. All groups treated with cadmium tended to maintain initial telomere length. Further investigations is needed to replicate the effect of cadmium and lead on telomere length as well as the interaction of cortisol with these metals.

1273 Increased Expression of CD44 in Cadmium and Arsenite Transformed UROtsa Cells

D. B. Osowski[2], A. Slusser-Nore[2], E. R. Evanoff[2], S. Somji[2], S. H. Garrett[2], D. A. Sens[2] and J. R. Dunlevy[2]. [1]*Basic Sciences, University of North Dakota, Grand Forks, ND and* [2]*Pathology, University of North Dakota, Grand Forks, ND.*

Heavy metals such as arsenite and cadmium are known carcinogens that aid in the development of bladder cancer. This laboratory has shown that the exposure of UROtsa cells to either arsenite or cadmium can cause malignant transformation of UROtsa bladder cells grown in culture. Microarray analysis of the parent versus transformed cell lines showed increased expression of several mesenchymal markers, one being fibronectin. The goal of the present study was determine if CD44, a receptor for fibronectin, could be developed as a potential biomarker for diagnosis, or prognosis, of bladder cancer. For this purpose, Real Time RT-PCR was performed on the RNA samples isolated for the UROtsa parental cell line, the 6 arsenite, and 7 cadmium transformed cell lines. The data obtained indicated the mRNA expression levels were elevated in all of the arsenite transformed cell lines and cadmium transformed cell lines when compared to the UROtsa parental lines. Western Blot analysis and immunofluorescence confirmed the previous results by showing increased levels of protein in the transformed cell lines compared to the parent with the highest staining for CD44 detected in the As#3, As#5, and As#6 cell lines. Immunofluorescence studies directly correlated with the mRNA and protein analysis showing a relatively high level of CD44 at the plasma membrane with higher levels with several of the arsenite and cadmium cell lines. Overall, the results of the study show that CD44 is increased in all of the transformed urothelial cell lines. These results suggest that high expression of CD44 may be associated with tumor progression and could be used as a biomarker in the diagnosis and prognosis of bladder cancer.

1274 Cytotoxicity and DNA Damage Induced by Copper and Zinc Organometallics in the HC-04 Non-Cancer Human Liver Cell Line

D. Desaulniers, G.-H. Xiao and C. Cummings-Lorbetskie. *Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada.* Sponsor: R. Mehta.

"Data-poor" Cu- and Zn-organometallics are anticipated to undergo risk assessments in the coming years. To gain insight for chemical prioritization and toxicity based on structure activity relationship, nine chemicals [CuCl2; CuBr2; Cu(II)D-gluconate (CuDg); Cu(II) dimethyldithiocarbamate (CDDC); benzenediazonium,3-methyl-4-(1-pyrrolielinyl)-,trichlorozincate hydrate (BDMT); benzenediazonium, 4-chloro-2-nitro-, tetrachlorozincate(2-) (2:1) (BCNZ); benzoic acid, 4-(1,1-dimethylethyl)-, zinc salt (2:1) (BADZ); zinc phenolsulfonate (ZPS); zinc p-toluenesulfonate hydrate (ZTSH)] were compared to five structurally related chemicals [ZnCl2; Zn-diethyldithiocarbamate (ZDDC); Na-dimethyldithiocarbamate; phenolsulfonate; sodium p-toluenesulfonate]. 4-nitroquinoline-oxide and aflatoxin-B1 (AFB1) were used as genotoxic positive controls. The HC-04 non-cancer human liver cell line was treated for 24 h (up to 500 µM). DNA was measured as an index of cellular abundance and DNA damage was assessed by both gamma-H2AX and DNA unwinding assays. The results indicated no significant effect induced by CuCl2, CuBr2, CuDg, and BCNZ. The toxicity of the dithiocarbamates was enhanced by the addition of a Cu or Zn moiety, while that of the biocides phenolsulfonate and toluenesulfonate by a Zn moiety. The dye BDMT induced more toxicity than the dye BCNZ. Cellular abundance was a more sensitive endpoint than gamma-H2AX. BADZ, BDMT, CDDC, ZPS, ZTSH, ZnCl2, and ZDDC increased cellular abundance at low concentrations (<100 µM) and decreased it at higher concentrations associated with increases in gamma-H2AX abundance. Interestingly, low concentrations (<100 µM) of ZnCl2, ZPS, and ZTSH also increased gamma-H2AX, suggesting proliferation of cells with damaged DNA and increases in potential carcinogenic risk. Ultimately, this study can provide insights for chemical and study prioritization with a view to mitigate chemical hazards (Funded by the Chemical Management Plan, Health Canada).

1275 Dynamics of Zinc and Arsenite Binding to XPA Zinc Finger and PARP-1 Zinc Finger 1 Peptides

J. Huestis, X. Zhou, L. Chen, C. Feng, L. G. Hudson and K. J. Liu. *College of Pharmacy, University of New Mexico, Albuquerque, NM.*

Inhibition of DNA repair is an established mechanism of arsenic co-carcinogenesis, and may be perpetuated by the binding interactions between trivalent arsenicals such as arsenite and key zinc finger DNA repair proteins. Validated molecular targets of arsenite include the first C3H1 zinc finger domain of Poly-ADP-Ribose Polymerase-1 (PARP-1), and the C4 domain of Xeroderma Pigmentosum Complementation Group A (XPA). In order to gain understanding of the thermodynamic and kinetic parameters of arsenite interaction with these two zinc finger motifs, zinc and arsenite binding to corresponding synthetic model peptides were probed using fluorescence based experiments. Variable concentrations of peptide were combined in tris-buffered solution with a wide range of zinc and arsenite concentrations. For kinetic experiments, changing fluorescence levels were measured in real-time (kinetics mode), and half-lives were used to derive a rate equation for arsenite binding of each peptide. For thermodynamic experiments, sample solutions were incubated for 3 hours, followed by endpoint measurement of fluorescence intensity, in order to determine ligand concentrations necessary for half-maximal binding of the peptide. While XPAzf and PARP-1zf-1 display similar levels of arsenite binding at equilibrium, PARP-1zf-1 shows a potential kinetic advantage over XPAzf for arsenite binding, with a reaction rate constant for the formation of arsenite-PARP-1zf-1 approximately 3 times greater than for arsenite-XPAzf. However, the binding reaction of zinc with either peptide proceeds at a faster rate than arsenite. Most notably, zinc and arsenite showed comparable levels of equilibrium binding to XPAzf, as well as to PARP-1zf-1 at lower peptide concentration. This finding suggests that the relative concentrations of zinc and arsenite in a system may strongly influence which species predominates in zinc finger occupancy. The results also highlight the potential utility of zinc supplementation in mitigating the adverse effects of arsenite exposure *in vivo*.

1276 Coagulation/flocculation Processes Using Inorganic Chemicals as Treatment for Textile Effluents

C. T. Onwordi[1,2] and P. C. Onianwa[2]. [1]*Chemistry, Lagos State University, Lagos, Nigeria and* [2]*Chemistry, University of Ibadan, Ibadan, Nigeria.*

Textile industries generate large volumes of highly coloured and organically laden wastewater. The need to treat the wastewater prior to discharge into drains/water bodies is of almost importance. Bench scale treatment of textile effluent was carried out by chemical coagulation / flocculation process. Treatments with ferrous chloride, ferrous sulphate, Ferric chloride, Fenton's reagent, alum, and activated carbon were used singly and binary. The wastewater was characterized by chemical oxygen demand (COD) 482 mg O2/L; total suspended solids (TSS) 135 mg/L; total dissolved solids (TDS) 780 mg/L and pH 10.4. The treatment process was carried out for 30 days to establish the optimum results for the parameters investigated. Efficiency of treatment options for COD reduction was in the order: Fenton's reagent (90%) > ferrous chloride (87%) > ferrous sulphate (66%) > alum (61%) > activated carbon (47%), while significant TDS reduction was not achieved. The pH of the treated samples with the various treatment options gave an acidic pH value,

especially the Fenton's reagent. The singly use of the forenamed chemicals resulted in better treatment compared with the binary combination. Experimental data emanating from this study indicated that singly chemical treatment is more efficient, and better capable of elevating the water quality of treated wastewater of the textile industry. Fenton's reagent and ferrous chloride were the most efficient chemicals treatment options for organic pollutants in textile effluent.

PS 1277 Human Health Risk of Heavy Metal Levels in Periwinkle, *Tympanotonus fuscatus* (Gastropoda) from a Contaminated Near Shore Tropical Wetland

A. I. Usese[4], L. O. Chukwu[4], R. Naidu[2,3], M. M. Rahman[2,3], S. Islam[2,3] and E. O. Oyewo[1]. *[1]Nigerian Institute of Oceanography and Marine Research, Victoria Island, Lagos, Nigeria; [2]Center for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Mawson Lakes, South Australia, Australia; [3]Cooperative Research Centre for Contamination Assessment and Remediation of the Environment, University of New Castle, Callaghan, New South Wales, Australia and [4]Department of Marine Sciences, University of Lagos, Lagos, Nigeria.*

With the growing concern that metals accumulated in fish or shellfish muscle tissues may represent a health risk especially for population with high consumption rate, a commercially valuable gastropod, which forms an important source of protein and industry in most parts of Nigeria was assessed for human health risk associated with bioaccumulated metals level. The concentrations of metals (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in tissues was determined by an Agilent 7500c Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) while the health risk posed by the consumption of Tympanotonus fuscatus from the highly impacted areas of the Lagos lagoon wetland was estimated by calculating the target hazard quotient (THQs) and hazard index (HI). The concentration of examined metals and metalloids in tissues were below the permissible limits (mg/kg dry weight) issued by JECFA of FAO/WHO and ANZFA for shellfish. Furthermore, the results show that THQ of all analyzed metals were lower than guideline value of 1 (US EPA, 2011) except for Arsenic (THQ; 1.97 and HI of 3.37), signifying that a long-term or daily exposure at this level may likely cause non-carcinogenic health effects to adult consumers.

PS 1278 Genetic Susceptibility and Occupational Exposure to Beryllium

A. Weston[2], E. C. McCanlies[2] and K. Kreiss[2]. *[1]Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV and [2]Respiratory Health Division, National Institute for Occupational Safety and Health, Morgantown, WV; [3]Respiratory Health Division (Retired), National Institute for Occupational Safety and Health, Morgantown, WV.*

Beryllium is a light-weight metal with properties that make it suitable for an array of technological uses. However, it is toxic and can cause immunological sensitization and chronic lung disease. Genetic susceptibility to beryllium sensitization (BeS) and chronic beryllium disease (CBD) have been recognized. HLA-DPB1 alleles that code for glutamic acid (E) at codon 69 have been strongly associated with BeS and CBD. However, the relatively high prevalence of HLA-DPB1*E69 in the population together with overall low risk of adverse health outcomes in beryllium exposed workers precludes its use to identify persons at risk. Previous studies revealed negative surface charge in the binding groove associated with three pairs of mostly polymorphic amino acids were associated with increasing genetic risk. Examples of the highest risk alleles code for aspartate (D) at position 55 and E56; E26 and E69; and D84 and E85; these alleles have an overall charge of -9. Workers and former workers (N=854) of a major U.S. beryllium producer were recruited to provide DNA samples for genetic DNA-sequence analysis for HLA-DPB1 (with approval of the NIOSH Institutional Review Board). This was performed using three forward and four reverse allele-specific DNA primers. The sequencing revealed that several HLA-DPB1*E69 genotypes that have higher risk for BeS and CBD than do others. In our study population 11 workers had a specific high-risk genotype (containing a charge -9 allele), of these 73% had BeS (all of which had CBD). In contrast, 21 workers had a relatively low-risk HLA-DPB1*E69 genotype (containing charge -7 alleles), and of these 48% had BeS (4 of which [19%] had CBD). The data suggest that among persons with the HLA-DPB1*E69 genetic trait, those with alleles that have a charge of -9, are at significantly (p=0.03) higher risk than those with alleles that have a charge of -7.

PS 1279 Standardization of Exhaled Breath Condensate (EBC) Collection Using a Feedback Regulated Breathing Pattern

B. R. Winters[2], M. Madden[4], M. Angrish[6], M. Stiegel[3], T. Risby[1] and J. Pleil[6]. *[1]Johns Hopkins University, Baltimore, MD; [2]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC; [3]Duke Medical Center, Duke University, Durham, NC; [4]EPHD, NHEERL/ORD, US EPA, Chapel Hill, NC; [5]HEASD, NERL/ORD, US EPA, Research Triangle Park, NC and [6]ISTD, US EPA, Research Triangle Park, NC.*

Collection of exhaled breath condensate (EBC) fluid by cooling of expired breath is a potentially valuable approach for the detection of biomarkers associated with disease or exposure to xenobiotics. EBC is generally collected using unregulated breathing patterns, perceived to elevate variability of biomarkers and volume of EBC collected. We examined the effects of breath frequency (f) regulation and fixed tidal volume (TV) on the variability of pH, cytokine concentration, and volume of EBC collected. Subjects were instructed to breathe either at their own natural TV and f ("spontaneous (S)") or to breathe with a TV of 1 liter and a f of 10 breathes/min ("non-spontaneous" (NS)). Breath was collected for 6 or 10 minutes with up to 3 sequential samples. To assist subjects with maintenance of a NS breathing pattern, a Loccioni Gruppa device (Angeli di Rosora, Italy) provided auditory and visual cues for inhalation/exhalation duration and volume. Results indicated that EBC volume collected using the NS method for 10 minutes had lower variability (CV: ± 0.066) relative to the S breathing (CV: ± 0.152). There was no discernible difference in the EBC pH or variability of the pH between sampling time or pattern. Inflammatory cytokine concentrations were measured, however detection limitations hampered strict statistical evaluation. These data indicate that providing subjects with a NS breathing method reduces EBC volume variability and may yield more consistent samples with repeated collections for noninvasive biomarker analysis. [This is an abstract of a proposed presentation and may not reflect US EPA official policy]. This investigation was supported by a pre-doctoral traineeship (NRSA T32 ES007126) from NIEHS.

PS 1280 Malondialdehyde-Acetaldehyde (MAA) Adducted Surfactant Protein Induced Lung Inflammation Is Mediated Through Scavenger Receptor A (SRA)

M. Sapkota and T. A. Wyatt. *Environmental and Occupational Health, University of Nebraska Medical Center, Omaha, NE.*
Sponsor: M. L. McCaskill.

Persons with alcohol use disorders (AUDs) are two to three times more likely to smoke cigarettes. Co-exposure to cigarette smoke and alcohol leads to the generation of high concentrations of acetaldehyde and malondialdehyde in the lung. These aldehydes are highly reactive due to their electrophilic nature and once formed react with proteins through a Schiff base reaction to generate MAA adducts. In mice, an increase in pro-inflammatory chemokine KC and lung lavage neutrophils was observed when instilled with MAA for 3 weeks. Also previous studies in bronchial cells and macrophages have shown that scavenger receptor A (SRA/CD204) is a major receptor for MAA. But no studies have examined the role of SRA in MAA mediated lung injury. So the main objective of the study was to determine the role of SRA in MAA induced lung inflammation. To test this hypothesis, C57BL/6 WT and SRA KO mice were nasally instilled with 50 ug/mL of SPD-MAA for 3-weeks. After 3 weeks, BAL fluid was collected and total cell count as well as differential cell count was performed. Lung was also harvested, embedded in paraffin and sectioned. The lung tissue sections were stained for hematoxylin and eosin (H&E) and MAA adduct. BAL fluid was assayed for cytokines, chemokines and growth factors. Results showed that BAL cellularity was decreased in SRA KO mice as compared to WT following repetitive SPD-MAA exposure marked by a decreased influx of neutrophils. Inflammatory cells within the peri-bronchiolar region of the small airways were markedly decreased in SRA KO mice in comparison to WT mice. MAA adduct staining in the lung epithelium was decreased in SRA KO mice as compared to WT mice. In comparison to WT, a decrease in CXCL1 (KC), M-CSF, GM-CSF, G-CSF, MCP-1 and MCP-5 was observed in BAL fluid from SRA KO mice. All these results suggest that SRA is important for SPD-MAA induced lung injury. In the absence of SRA, there is less binding of MAA in lung columnar epithelium and decreased neutrophil influx in BAL and lung parenchyma. Overall, the data demonstrate that SRA/CD204 plays an important role in SPD-MAA induced inflammation in lung as evidenced by the diminished inflammation in the absence of this receptor.

1281 Persistent Increases in Inflammatory Cytokines, Akt, and MAPK/ERK Pathways After Inhalation Exposure of Rats to Libby Amphibole (LA) or Amosite: Comparison to Effects After Intratracheal Exposure to LA or Naturally Occurring Asbestos

S. H. Gavett[2], D. L. Andrews[2], U. P. Kodavanti[2] and J. M. Cyphert[1]. [1]*Duke University, Durham, NC and* [2]*US EPA, Research Triangle Park, NC.*

Human exposure to LA and other mined or processed asbestos increases risk of lung inflammation, fibrosis, and cancer. Health risks from exposure to naturally occurring asbestos (NOA) are not as well-understood. Mechanisms of long-term toxicity were compared in male F344 rats exposed by nose-only inhalation (IH) or intratracheal (IT) instillation. Responses to IH LA (1.0, 3.3, or 10.0 mg/m3), amosite (AM; 3.3 mg/m3, as positive referent group), or air (all 6 hr/d, 5 d/wk, 13 wk) were evaluated 1 d, 1 mo, and 3 mo post-exposure. Responses to IT LA and NOA samples Sumas Mountain chrysotile (SM), El Dorado Hills tremolite (ED), and Ontario ferroactinolite (ON) (all at 0.5 or 1.5 mg/rat), or dispersion media (DM) only, were evaluated 1 d and 3 mo post-IT. Inflammatory cytokines, Akt, and MAPK/ERK pathway proteins were examined in lung tissue. Three mo after IH LA at 10.0 mg/m3, MAPK/ERK components (STAT3, MEK1/2) were significantly elevated 40-50% over air control. One day after IT LA or SM, STAT3, MEK1/2, and ERK1/2 were increased 2-5x over DM control. These IT effects were not present (MEK1/2, ERK1/2) or strongly attenuated (STAT3 for LA) at 3 mo post-exposure. Akt pathway components (S6RP, Akt) were increased 2x over air control 3 mo after 10.0 mg/m3 IH LA. One day post-IT, S6RP was increased 4x by 1.5 mg LA and 9x by 1.5 mg SM, and Akt was increased 2x by SM. By 3 mo post-IT, only Akt was significantly increased (2x by SM). TNF-α, IL-1β, and CXCL1 were persistently increased 1 d to 3 mo after IH AM or LA. These targets were also increased 1 d post-IT LA and SM, but by 3 mo only CXCL1 and TNF-α remained elevated (for LA and ED). At 15 mo post-IT and 18 mo post-IH, LA promoted lung fibrosis and tumor development. We conclude that LA inhalation causes more persistent inflammation and activation of growth factor pathways than IT exposure, which may contribute to lung disease long after initial exposure. Pro-inflammatory effects of IT SM and LA were greater than those of ED and ON. (This abstract does not represent US EPA policy.)

1282 Inflammatory Effects of Acrolein, Crotonaldehyde and Hexanal on Air-Lifted Primary Bronchial Human Epithelial Cells

A. M. Dwivedi, L. Ernstgård, G. Johanson and L. Palmberg. *Work Environmental Toxicology, Karolinska Institutet, Stockholm, Sweden.*

Acrolein (ACR) and crotonaldehyde (CRO) are formed during incomplete combustion and are found in e.g. cigarette smoke and restaurant kitchens. Hexanal (HEX) is emitted from wood pellets, MDF board and used as flavoring additive in cigarettes. The aim of the study was to compare the inflammatory effects of aldehydes in an air-liquid interface (ALI) exposure system with on air -lifted primary bronchial human epithelial cells (PBEC). The air-liquid interface (ALI) exposure system was used to achieve a closer resemblance with inhalation exposure *in vivo*. PBEC were obtained from multiple donors and cultured and grown in the ALI system. After 8-10 days at ALI, these cells were exposed to ACR (0.05, 0.1 and 0.5 ppm), CRO (0.5, 1.0, 2.0 and 5ppm), HEX (5, 10, 20 and 50 ppm) or clean air for 30 minutes. Samples of basal and apical media were collected at 8 h and 24 h after exposure and analyzed for Interleukin-8 (IL-8) and Matrix Metalloprotein-9 (MMP-9) by enzyme-linked immunosorbent assays (ELISA). Cell viability was tested at the same time points by trypan staining. Reduced cell viability (70-80% cell survival) was only seen at the highest tested aldehyde concentrations. Both ACR and CRO caused elevated IL-8 levels in basal media after 8 h and more so after 24 h. The IL-8 levels tended to increase with concentration but started to decrease at the highest tested concentrations. A different pattern was seen in apical media, with an inverse relation between aldehyde and IL-8 concentrations. None of these trends were statistically significant. There were no effects of HEX on IL-8. No clear effects were seen on MMP-9 for any of the aldehydes. Our study (1) shows that the ALI system can be used to study the inflammatory response of aldehydes, (2) supports that the potency to induce an inflammatory response follows the order ACR>>CRO>>HEX, and (3) indicates that the IL-8 response patterns seem to differ between the basal and apical side in the ALI system.

1283 Dung Biomass Smoke Exposure Attenuates Immune Responses to Toll-Like Receptor Ligands in Airway Epithelial Cells and Mice

C. E. McCarthy[2], P. F. Duffney[2], T. H. Thatcher[2], R. P. Phipps[2] and P. J. Sime[2]. [1]*Environmental Medicine, University of Rochester, Rochester, NY and* [2]*Medicine, Pulmonary Diseases and Critical Care, University of Rochester, Rochester, NY.*

Worldwide, 3 billion people use biomass fuels, including animal dung, for cooking and heating their homes. According to the World Health Organization, over 4 million premature deaths every year are attributed to biomass smoke inhalation. Women and children, who often spend the most time around cooking fires, are at high risk of biomass smoke exposure. Epidemiological studies associate biomass smoke with an increased risk for respiratory infections, and nearly half of the pneumonia-related deaths in young children are related to biomass smoke. However, there is currently little experimental data examining how dung biomass smoke exposure impacts host-pathogen interactions. We hypothesized that dung biomass smoke impairs innate immune responses in the lung. To test this hypothesis, we generated dung biomass smoke using a novel, automated system. Primary human small airway epithelial cells (SAEC) were grown on transwell inserts, moved to the air-liquid interface, and exposed to air or dung smoke. Subsequently, cells were stimulated with Poly I:C (a Toll-like (TLR)-3 ligand) or MALP-2 (a TLR-2 ligand). Cytokine production was assessed by ELISA, IFN-β gene expression was measured by qPCR, and interferon levels were determined using ISRE-reporter cells. For *in vivo* experiments, mice were exposed to dung biomass smoke 2x1 hour per day (TPM = 50 mg/m3) for 2 weeks. After smoke exposure, mice were treated with LPS (a TLR-4 ligand) via inhalation. Inflammatory mediators and proteins were measured in the lung and BALF by ELISA and Western blot. Histology was used to examine lung inflammation. We found that SAEC exposed to dung biomass smoke had heightened IL-8 secretion in response to MALP-2 and attenuated upregulation of cytokines and interferon levels after Poly I:C treatment. Mice exposed to dung biomass smoke for 2-weeks had reduced levels of neutrophil chemoattractants and more inflammation after LPS inhalation. Our results show that dung biomass smoke exposure dysregulates pulmonary innate immune responses to pathogen-associated molecular patterns. We propose that impaired innate immunity due to dung biomass smoke inhalation may contribute to an increased risk and severity of respiratory infections, especially in vulnerable populations living in developing countries.

1284 Pulmonary Responses Following Inhalation of Sand Dust Collected From Hydraulic Fracturing Operations

J. S. Fedan[1], J. A. Thompson[1], B. Y. Farris[2], W. McKinney[1], A. M. Cumpston[1], M. C. Jackson[1], K. A. Russ[1] and J. R. Roberts[1]. [1]*Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, Morgantown, WV and* [2]*Pathology and Physiology Research Branch, West Virginia University, Morgantown, WV.*

Workers at gas well fracking operations are exposed to fine fracking sand dust (FSD) which is generated from the movement of sand as it is prepared for injection into the well as a proppant. The worker exposures to FSD have been reported to exceed the exposure limits for crystalline silica promulgated by OSHA, NIOSH and ACGIH. Characterization of the FSD indicated that 88% of the particles were ≤2 µm, very low in Si•, SiO•, or SiOO• radicals, and >95% quartz. We investigated the effects of FSD inhalation on the lung using Sprague-Dawley rats. Animals were exposed in whole-body chambers to 10 or 30 mg/m3 FSD collected from a fracking site or filtered air (control) for 6 h/d for 4 d. Measurements were made at 1, 7 and 27 d post-exposure. Neither 10 nor 30 mg/m3 FSD affected basal pulmonary function (lung resistance, RL; dynamic compliance, Cdyn) at any post-exposure time. At 10 mg/m3 FSD Cdyn responses to inhaled methacholine (MCh) were decreased 7 d post-exposure. At 30 mg/m3 FSD reactivity to MCh (RL) was increased at 7 and 27 d post-exposure. No other changes in reactivity to MCh were observed. Lung injury, measured as lactate dehydrogenase activity in bronchoalveolar lavage (BAL) fluid, was not increased following exposure to either dose of FSD. Neutrophil influx into the lung, an index of inflammation, increased at 7 d following exposure to 10 mg/m3 FSD, whereas neutrophils were elevated at 1 d post-exposure following inhalation of 30 mg/m3 FSD. BAL phagocyte oxidant production was increased at 7 d following inhalation of 30 mg/m3 FSD. Inflammatory responses were resolved by 27 d post-exposure for both doses of FSD. The results indicate that a four-day exposure of rats to FSD elicits modest changes in pulmonary function and lung inflammation.

1285 Activation and Desensitization of Transient Receptor Potential Vanilloid-1 (TRPV1) Variants by Coal Fly Ash Particles and Associations with Asthma Symptom Control Metrics

C. Rice, C. Stockmann, E. Romero, D. Shapiro, J. Veranth and C. Reilly. *University of Utah, Salt Lake City, UT.*

People with asthma are sensitive to many inhaled irritants including air pollution and capsaicin. Transient receptor potential vanilloid-1 regulates the acute sensitivity of people to selected inhaled irritants and may shape asthma phenotypes. We hypothesized that polymorphic variants of TRPV1 would be uniquely responsive to traditional soluble (capsaicin) and atypical insoluble particle (coal fly ash) agonists. Furthermore, these changes would manifest as differences in asthma severity and control. The TRPV1 I315M and T469I variants were more responsive to agonists, as measured by calcium flux in HEK-293 cells transiently transfected with the variants, due to an apparent increase in expression in cells compared to wild-type TRPV1. The I585V variant was inherently less responsive to coal fly ash particles. A patient cohort of asthmatic children was genotyped to determine prevalence of the 3 TRPV1 variants. All three variants affected asthma control, with I585V consistently being associated with worse asthma control, as evidenced by increased symptom frequency, greater impact of asthma on normal activities, and the frequency of inhaler use and healthcare visits for asthma exacerbations. The basis of this phenomenon may be attributable to a combination of increased TRPV1 expression among people with asthma and limited agonist-induced desensitization of TRPV1 by particles and by the I585V variant. Among smoke exposed subjects, carriers of the I315M and T469I variants, but not I585V, experienced poorer asthma control. These findings indicate that TRPV1 polymorphisms contribute to an individual's relative sensitivity to selected inhaled irritants such as air pollution or smoke. Furthermore, identifying TRPV1 variations could hold promise for personalized asthma care. Support: ES017431.

1286 Toxicological Characteristics of Russian Anthophyllite: Chemical Composition and Cancer Potency Considerations

A. Korchevskiy and J. Rasmuson. *Research & Development, Chemistry & Industrial Hygiene, Inc., Wheat Ridge, CO.*

Rationale and Scope. Toxicological characteristics of anthophyllite fibers are not well known, in spite of the fact that anthophyllite was actively mined in the United States and other countries. An in-depth mineralogical study was performed and various approaches were applied to evaluate mesothelioma and lung cancer potency factors for this mineral. Research Approach. A suspect amphibole specimen was obtained in the Russian Federation where the mineral is on public display in a local park. Characterization of this specimen was accomplished using Transmission Electron Microscopy (TEM) employing select area electron diffraction and zone axis indexing, Mössbauer spectroscopy to determine the FeO to Fe2O3 ratio and electron probe micro-analysis (EPMA) for major elemental composition. Regression modeling was used to reconstruct potency factors of the mineral based on its chemical composition. Study's Results. The specimen was determined to be anthophyllite asbestos, with an average SiO2 content of 58%, MgO of 27%, Fe2O3 2.3 % and FeO 0f 8%. An average fiber aspect ratio of 25 was also determined. The average mesothelioma potency of the mineral was estimated as RM=0.05 % per f/cc-year based on measured physical and chemical characteristics. Available data for mesothelioma incidence in the district of anthophyllite mining in Russia suggest a potency level at 0.056% per f/cc-year (95 % CI 0.008-0.13). Lung cancer potency of Russian anthophyllite can be estimated as RL=0.99% per f/cc-year, higher than for Libby amphibole at 0.8% per f/cc-year, but lower than for amosite, 1.89% per f/cc-year. Conclusions. Russian mining of anthophyllite asbestos is not well-known. In spite of apparent closure of anthophyllite mining operations, its input in mesothelioma and lung cancer mortality in the country should not be ignored. Utilization of the proposed empirical model for cancer potency factors (depending on chemical and physical characteristics of the fibers) yields acceptable results if compared to levels expected from epidemiological studies. Quantitative estimation of amphibole potency can be applied in retrospective risk assessments and in analysis of toxicological modes of action.

1287 Polyhexamethyleneguanidine-Phosphate Induce Epithelial-to-Mesenchymal Transition in Human Lung Adenocarcinoma Cell Line, A549

D. Y. Shin, H. R. Kim, L. Seung Mee and K. H. Chung. *Sungkyunkwan University, Suwon, Korea, Republic of.*

In 2011, lung disease characterized by massive fibrosis occured in Korea. Epidemiological studies confirmed the relationship between the use of humidifier disinfectants and the lung disease. One of the component of humidifier disinfectant, polyhexamethyleneguanidine phosphate (PHMG-p), induced inflammatory and fibrotic responses in previous *in vivo* and *in vitro* studies. However, the detailed mechanism of fibrotic responses is unknown yet. Epithelial-to-mesenchymal transition (EMT) is a process that can occur in fibrosis, carcinogenesis, etc. Under EMT, epithelial cells lose their epithelial properties like polarity, cell-cell adhesion and become individualized cell and gain mesenchymal markers. Though EMT is important mechanism of fibrosis, the effect of PHMG-p in EMT is not clearly investigated. In this study, the effect of PHMG-p on alveolar epithelial cell line was investigated using A549 cell line. First, WST-1 assay was conducted to measure the cytotoxicity of PHMG-p. Second, morphological alteration after PHMG-p treatment was observed by microscopy. Third, changes of protein expression of E-cadherin and α-SMA (alpha smooth muscle actin) were investigated. Lastly, to study the mechanism of PHMG-p-induced EMT, gene expressions of EMT-related genes were measured through real-time PCR. PHMG-p showed significant reduction of cell viability. When treated for 72 hours, PHMG-p induced morphological change of A549 cell lines to spindle-like, elongated shape. Western blotting data showed that PHMG-p induce reduction of E-cadherin, which is marker of epithelial cells and increase of α-SMA, which is marker of mesenchymal cells. The gene expression of ZEB2 was increased significantly, which suggest that transcription factor ZEB2 might be involved. In conclusion, PHMG-p induce EMT in A549 cell line, and ZEB2 can be involved in the PHMG-p induced EMT.

1288 Naphthalene DNA Adduct Formation in *Ex Vivo* Rodent Tissues

S. A. Carratt[3], B. A. Buchholz[1], X. Ding[2] and L. S. Van Winkle[3]. *[1]CAMS/LLNL, Livermore, CA; [2]SUNY, Albany, NY and [3]UC Davis, Davis, CA.*

Naphthalene (NA) is a respiratory toxicant and possible human carcinogen. Human epidemiology data are unclear on the long-term effects of NA exposure and risk assessment relies heavily on animal data. Recurrent cycles of cytotoxicity and proliferation are thought to be the driving force behind formation of mouse lung tumors and rat nasal tumors; however, the formation of DNA adducts from NA metabolites has not been ruled out and could impact the decision to evaluate NA as a genotoxic agent. This study evaluated DNA adduct formation in target tissues for carcinogenesis in the National Toxicology Program's chronic bioassays: female mouse airway epithelium and male rat nasal epithelium (respiratory, olfactory). Metabolically active epithelial tissue was isolated and incubated in explant culture with 14C-labeled NA (0, 2.5, 25, 250 uM) for 1 h. The tissue was then rinsed 12-15 times to remove unbound NA; DNA was isolated and prepared for 14C-AMS (accelerator mass spectrometry) analyses. Male rat airway, which is not susceptible to tumor formation by NA, was used as a negative control. Benzo(a)pyrene (25 uM), a known carcinogen and DNA adductor, was the positive control. We found the highest level of *ex vivo* formed NA-DNA adducts in mouse airway, followed by rat airway and nasal olfactory tissue, in a manner corresponding with the previously reported, differing rates of *in vitro* NA bioactivation at these sites. We conclude that NA is capable of forming DNA adducts in target tissues of NA carcinogenesis in mice and rats. Future experiments will determine adduct structure and examine adduct stability, both properties may impact the carcinogenic potential of the detected DNA adducts. This work performed in part under the auspices of the US Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. Funded by T32 ES007059, R01 ES020867, ES020867S1 and DOD LC130820.

1289 Reactive Oxygen Species-induced DNA Effects of Peripheral Blood Mononuclear Cells Isolated From Rats After Pulmonary Exposure to Welding Fume

M. Shoeb, V. Kodali, A. Erdely, T. Meighan, R. Salmen, T. Eye, P. C. Zeidler-Erdely, B. Farris, J. R. Roberts and J. M. Antonini. *NIOSH, Morgantown, WV.*

Welding fume is a complex mixture of different potentially cytotoxic and genotoxic metals, such as Cr, Mn, Ni, and Fe. The pulmonary effects of welding fume have been well-described; however, less is known about the extra-pulmonary responses. The objective of the study was to assess the systemic effects of welding fume by examining molecular and cellular changes of isolated peripheral blood mononuclear cells (PBMC) in a rat model. Male Sprague-Dawley rats were treated by intratracheal instillation (IT) with 2.0 mg/rat of gas metal arc-mild steel (GMA-MS) and manual metal arc-stainless steel (MMA-SS) welding fume. Vehicle controls received sterile saline by IT. At 4 h, 14 h, 72 h, and 10 d, bronchoalveolar lavage (BAL) was performed to assess lung toxicity. Whole blood was collected, PBMC were isolated, and the production of reactive oxygen species (ROS) and DNA alterations in PBMC were assessed by measuring 4-hydroxylnonenal protein adduct (P-HNE) formation using fluorescence microscopy and DNA methylation and telomere length, respectively. Metal composition of the two fumes was different: MMA-SS (41% Fe, 29% Cr, 17% Mn, 3% Ni) versus GMA-MS (85% Fe, 14% Mn). BAL indicators of lung injury and inflammation were increased with MMA-SS treatment compared to other exposures at 14 h, 72 h, and 10 d after treatment. ROS generation and P-HNE adduct formation increased at 14 h in the PBMC recovered from the MMA-SS group compared to other groups. Furthermore, an increase in DNA methylation in PBMC was measured as early as 4 h after MMA-SS treatment, whereas variable responses in telomere length were observed when comparing the groups at the different time points. These findings suggest that genotoxic metals in MMA-SS fume (e.g., Cr and Ni), that are absent in the GMA-MS fume, may enhance lung toxicity, as well as generation of oxidative-stress markers and DNA alterations in PBMC.

1290 DNA Damage and Senescence Result in Epithelial Dysfunction and Pulmonary Fibrosis

T. Beach, C. J. Johnston, A. M. Groves, J. P. Williams and J. N. Finkelstein. *University of Rochester, Rochester, NY.*

Data from human subjects has indicated Radiation Induced Lung Injury (RILI) leading to pulmonary pneumonitis and/or fibrosis occurs in 43% of all cases following radiation treatment (RT) of 15Gy or greater to the lung, with severity of injury being dependent on the lung volume irradiated and dose received. While pneumonitis is largely treatable with corticosteroid regimens, radiation induced pulmonary fibrosis (RIPF) is generally considered a life threatening, irreversible event. DNA damage as a result of radiation treatment is known to induce apoptosis, and has long been theorized to induce senescence among the surviving cell population. We have reported a decline in club cell populations and fluctuations in the immune populations in response to RT, and recent evidence suggests a decline in the type 2 alveolar epithelia as well. It is hypothesized that radiation induces persistent DNA damage and senescence in whole lung tissue, resulting in a senescence associated secretory phenotype (SASP) and type 2 epithelial population decline associated with the onset of pulmonary fibrosis. A model consisting of 18 month aged C57Bl/6J mice exposed to 12.5 Gy whole lung irradiation via a Cesium137 gamma radiation source was compared with non-irradiated age matched controls. SASP cytokines Il-1α, Il-6, CXCL-1 (KC), Surfactant Protein C (SPC) and p21 abundance was assessed in whole long tissue by rt-PCR. Changes in stromal cell populations were assessed by flow cytometry and histology, with p21 and senescence associated beta-galactosidase (SA-βgal) staining for senescence indication by histological examination. Irradiated lung displays morphology indicative of pulmonary fibrosis, and a migratory pattern of senescence expression. Whole lung tissue indicates increases in p21, decline of SPC mRNA abundance, as well as induction of SASP cytokines Il-1α, Il-6, and CXCL-1 (KC) following RT. Expression of p21 and SA-βgal is potentially linked to decline in epithelial cell subtypes within the lung which we believe to be both pro-inflammatory and pro-fibrotic. Ultimately this research may lend insight to the role senescence plays in the disruption of cell populations and signaling leading to pulmonary disease, and yield insight to therapeutic interventions for radiation induced lung injury. Supported by: R01 AI101732-01, U19AI091036, P30 ES-01247 and ES T32 07026.

1291 Gender Differences in Murine Pulmonary Responses Elicited by Nano-Crystalline Cellulose

A. A. Shvedova[2,7], E. R. Kisin[2], N. Yanamala[2], M. T. Farcas[2], A. L. Sayre[2], D. W. Gutkin[6], P. M. Fournier[3], A. Star[3], R. Reiner[5], S. Halappanavar[4] and V. E. Kagan[3]. *[1]Chemistry, University of Pittsburgh, Pittsburgh, PA; [2]EAB, NIOSH, Morgantown, WV; [3]Environmental & Occupational Health, University of Pittsburgh, Pittsburgh, PA; [4]Environmental and Radiation Health Sciences Directorate, Health Canada, Ottawa, ON, Canada; [5]Forest Product Laboratory, USDA Forest Service, Madison, WI; [6]Pathology, UPMC, Pittsburgh, PA and [7]Physiology & Pharmacology, WVU, Morgantown, WV.*

The emerging field of nanotechnology attracts considerable attention to special properties of nanoparticles that are utilized in a number of novel products. Cellulose-based materials have been used for centuries to manufacture different goods derived from forestry and agricultural sources. In the growing field of nano-cellulose applications, its uniquely engineered properties (e.g. mechanical, thermal, rheological and optical) are instrumental for inventive products coming to competitive markets. Cellulose nanocrystals (CNC) is a crystalline form of cellulose obtained from different cellulose sources (bacterial, alga, wood pulp, cotton, etc.) by acid hydrolysis. CNCs are already produced on a large scale as nanofillers in polymer composites, building materials, cosmetics, food, and the drug industry. The high aspect ratio and stiffness of CNC may cause similar pulmonary toxicity as carbon nanotubes and asbestos thus posing a negative impact on public health and the environment. The present study was undertaken to investigate the pulmonary outcomes induced by repeated exposure to respirable CNC. C57BL6 female and male mice were treated by pharyngeal aspiration with CNC (40 µg/mouse) two times a week for three weeks. Exposure to respirable CNC caused pulmonary inflammation and damage, cytogenic alterations assessed by frequency of bi- and micro-nucleated cells in BAL, accelerated oxidative stress, elevated TGF-β, collagen increase in lung and impaired pulmonary functions. Notably, these effects were markedly more pronounced in female compared to male mice. Moreover, gender differences in responses to pulmonary exposure to CNC were also detected at the level of global RNA expression as well as in cytokine/chemokine inflammatory reactions. Overall, our results indicate that there are considerable gender differences in responses to respirable CNC with a higher pulmonary toxicity observed in female mice.

1292 Immunomodulatory and Epigenetic Effects of Resveratrol in the Attenuation of SEB-Induced Acute Lung Injury

H. F. Alghetaa[1,3], E. Zumbrun[3], A. K. Mohammed[1,3], D. Elliot[3], P. Nagarkatti[3] and M. Nagarkatti[2,3]. *[1]University of Baghdad - College of Veterinary Medicine, Baghdad, Iraq; [2]Veterans Affairs, Columbia, SC and [3]Pathology, Microbiology and Immunology, University of South Carolina - School of Medicine, Columbia, SC.*

Acute lung injury (ALI) is a common cause of death among people worldwide. Recently, plant-derived compounds such as resveratrol (RES), which has antioxidant and anti-inflammatory properties, has been explored as a possible treatment against inflammatory diseases. In this study, we explored the role of RES in suppressing inflammation induced by an inhaled super-antigen, staphylococcal enterotoxin B (SEB). To this end, mice were pretreated with RES or vehicle and the ALI was induced by dual-dose administration of SEB. All SEB exposed mice pretreated with vehicle succumbed within 120 hours, while 100% of mice pretreated with RES survived. CD3, CD4, CD8 and NK+CD3 lymphocyte subsets in the lungs of SEB-exposed mice treated with vehicle were significantly higher than RES treated mice. Pulmonary function was evaluated and showed impairment in the vehicle treatment group that was restored with RES treatment. Histopathological analysis and the vascular leak test demonstrated that SEB caused a statistical increase in infiltrating cells into lung parenchyma, likely explaining the clinically observed dyspnea and anoxia. Interestingly, cell cycle analysis revealed that infiltrating cells from the lung were paused in G2/M phase in both mice groups. Total RNA was extracted from lung infiltrate for microRNA (miRNA) microarray and several miRNAs were altered upon RES treatment. Downregulation of miR-193a-3p, which targets TGF-beta was confirmed by real-time PCR. Importantly, TGF-beta upregulation is known to result in increased T-reg cells and reduced activated CD4+ cells, as seen with RES treatment. We conclude that RES treatment protects against super-antigen induced inflammation by immobilizing T-cells and thereby decreasing vascular endothelial dysfunction at the site of exposure, possibly by acting through miRNAs. (Supported by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357).

1293 *In Situ* Metabolite Profiling of Remodeled Arteries in Pulmonary Arterial Hypertension Using Innovative Mass Spectrometry Imaging Tools

H. Gregory[3], S. J. Dumas[2], R. Legouffe[3], G. Picard De Muller[3], P. Dorfmüller[2], E. Fadel[2], M. Humbert[2], J. Stauber[3], S. Cohen-kaminsky[2], F. Pamelard[3] and D. Bonnel[3]. *[1]Imabiotech, Loos, France; [2]INSERM UMR-S 999, Univ Paris-Sud, LabEx LERMIT, Le Plessis Robinson, France and [3]MS Imaging Dept, Imabiotech, Loos, France.*

Pulmonary arterial hypertension (PAH) is associated to increased pulmonary arterial pressure, resulting in right heart failure and death. Understanding vascular remodeling pathways is a challenge for discovering novel therapeutic and diagnostic tools. Direct *in situ* metabolite profiling of pulmonary arteries using mass spectrometry imaging (MSI) combined with the Multimaging™ analysis tool could be a pertinent strategy to better understand PAH pathways. 1) *In situ* detection and quantification of metabolites in pulmonary arteries in order to determine the molecular signature of vascular remodeling. 2) Assessment of Multimaging™ in the context of multiple comparisons of complex tissues. Human frozen lung samples from the tissue bank of the French Referral Center for PAH were cut in 10µm-thick sections and mounted onto ITO conductive slides. Analysis was performed with a SolariX FTICR 7.0T Mass Spectrometer (Bruker Daltonics) at a 30 µm spatial resolution combined to the Multimaging™ analysis tool, allowing selection of areas containing vessels using contrast ions, and automated analysis of metabolites. Several metabolites showed differential levels between healthy and PAH samples with some m/z signals remaining to be characterized. Typical molecular signatures of the different types of vascular lesions were highlighted. For example, cADP (Cyclic ADP) ribose (key mediator of hypoxic pulmonary vasoconstriction) and ADP ribose, two metabolites belonging to the same pathway, were overexpressed in PAH lesions. MSI combined with Multimaging™ allowed the discovery of molecular signature of vascular remodeling in PAH. This strategy could open new avenues in understanding complex diseases on the basis of multiple tissue comparison.

1294 Inhibition of Lactate Dehydrogenase Prevents Radiation-Induced Fibrotic Responses in the Lung

J. L. Judge[2], J. P. Williams[2], R. P. Phipps[2], P. J. Sime[2] and R. M. Kottmann[2]. *[1]Environmental Medicine, University of Rochester, Rochester, NY and [2]Medicine, University of Rochester, Rochester, NY.*

Exposure of the lung to ionizing radiation can result in pulmonary fibrosis. Unfortunately, few treatments are available for this chronic, progressive disease. Pulmonary fibrosis is characterized by an accumulation of extracellular matrix proteins secreted by differentiated lung fibroblasts called myofibroblasts. Although the mechanisms surrounding this process are not well understood, one possible mechanism involves activation of the pro-fibrotic cytokine transforming growth factor-beta (TGFβ). Our lab has shown that the metabolite lactate activates extracellular latent TGFβ via a reduction in extracellular pH. We recently published that lactate dehydrogenase-A (LDHA), the enzyme that produces lactate, is up-regulated in patients with radiation-induced pulmonary fibrosis. Furthermore, genetic inhibition of LDHA prevented radiation-induced myofibroblast differentiation via inhibition of TGFβ activation. We have yet to identify whether LDHA is a valid therapeutic target in radiation-induced fibrosis *in vivo*. We hypothesize that LDHA inhibition *in vivo* will prevent radiation-induced pulmonary fibrosis. To test this hypothesis, C57Bl/6 mice were exposed to 5 Gray total body radiation and 10 Gray thoracic radiation from a 137Cs source to induce pulmonary fibrosis. Starting 4 weeks post-radiation, mice were treated with 5 mg/kg of the LDH inhibitor Gossypol or vehicle daily until sacrifice at 26 weeks post-radiation. Collagen content (hydroxyproline assay), mRNA, and micro (mi) RNA expression were measured in lung homogenates. Radiation induced an increase in LDHA, collagen I and III, fibronectin and TGFβ mRNA expression as well as total lung hydroxyproline content. Fibrosis and the associated markers were inhibited by Gossypol treatment. Radiation also down-regulated miR-338-3p, an LDHA target miRNA, and Gossypol rescued this down-regulation. Our results demonstrate that inhibition of LDHA protects from radiation-induced pulmonary fibrosis, which was associated with altered miRNA expression, suggesting a role for epigenetic regulation of LDHA following radiation.

1295 Acetaminophen Attenuates House Dust Mite Induced Allergic Airway Disease in Mice

G. J. Smith[3], R. S. Thrall[2], M. M. Cloutier[1], J. E. Manautou[3] and J. B. Morris[3]. *[1]Connecticut Children's Medical Center, Hartford, CT; [2]Department of Immunology, University of Connecticut Health Center, Storrs, CT and [3]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT*. Sponsor: J. Morris.

Epidemiological evidence suggests that APAP (N-acetyl-para-aminophenol, APAP) may play a role in the pathogenesis of asthma, likely through pro-oxidant mechanisms. Our previous data demonstrate that APAP, at moderate doses, acts as a pro-oxidant in the airways and enhances the airways response to a known oxidant asthma risk factor, environmental tobacco smoke. However, no studies have investigated the direct effects of APAP on the development of allergic inflammation. In order to determine the likelihood of a causal relationship between APAP and asthma pathogenesis, we explored the effects of APAP on inflammatory responses in a murine house dust mite (HDM) model of allergic airway disease. The HDM model consisted of once daily intranasal instillations for up to two weeks. APAP was administered 1 hour prior to HDM during either week 1 or 2. Unexpectedly, the effects of HDM treatment were substantially diminished in APAP treated groups compared to controls as indicated by markedly reduced airway inflammation (as assessed by bronchoalveolar lavage), lower cytokine expression in lung tissue, and less perivascular and peribronchiolar immune cell infiltration (as assessed by histopathology). The anti-inflammatory effect of APAP was not abrogated by an inhibitor of cytochrome P450 metabolism, suggesting that the effect was due to the parent compound or a non-CYP450 generated metabolite. Taken together, our studies do not support the biological plausibility of the "APAP hypothesis" that APAP use may contribute to the causation of asthma. (Supported by the University of Connecticut President's Research Award)

1296 Sensitivity and Resistance to Lung Inflammatory and Pathological Changes in Carbon Nanotube Exposures Among Inbred Mouse Strains

E. A. Frank[2], V. S. Carreira[2], D. R. Prows[1] and J. S. Yadav[2]. *[1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH and [2]University of Cincinnati, Cincinnati, OH.*

Carbon nanotubes (CNTs) are forecasted to become a highly prevalent material in many technological and industrial fields, including civil engineering, energy production, aeronautics, and biotechnology. This is reflected in the striking increase in workplaces involved in the manufacture and handling of CNTs, where a rapidly growing number of occupational workers risk exposure to CNTs via inhalation of airborne fibrous particulates. Many recent studies have worked to characterize the toxicological effects and mechanisms of exposure to CNTs using inbred mouse models, but comparative studies investigating strain-to-strain sensitivity to toxic CNT effects have not been reported and the extent to which genetic background may influence sensitivity or resistance to the effects of CNT exposures has not been demonstrated. In this study, we surveyed four inbred mouse strains (C57Bl/6, BALB/c, C3H/HeJ, DBA/2) and compared the severity of their responses in terms of lung tissue pathology and cellular infiltration over 1-, 14-, or 28-day pulmonary exposures to 4 mg/kg/bw CNTs or vehicle (n=5) delivered via oropharyngeal aspiration. We found that C57Bl/6 mice developed notably more severe lung pathology after 14 and 28 days which consisted of granulomatous inflammation, type II pneumocyte hyperplasia, lymphoid tissue hyperplasia, and alveolar wall thickening compared to DBA/2 mice. In contrast, bronchoalveolar lavage samples from DBA/2 mice demonstrated far more pronounced increases in cell infiltration, which consisted primarily of neutrophils and monocytes/macrophages, compared to C57Bl/6. Follow up work using F1 hybrids (B6D2F1) demonstrated a range of sensitivities falling between that of the two parent strains, indicating that several genetic loci likely contribute to the polarity between responses of either strain. Collectively, the results: 1) demonstrate that genetic background may influence the severity of toxicological responses to CNTs, indicating that inter-individual susceptibility dependent on gene-environment interactions in human exposures likely exists, and; 2) identify sensitivity and resistance among commonly used mouse strains, which may inform selection of experimental models in future toxicological work.

1297 Changes in Vascular and Exhaled Breath Condensate Nitrite Following Acute Traffic Pollution Exposure Using Oral Nitrite Prophylaxis via Beet Juice

H. Kipen[2], C. Guo[1], R. Laumbach[3], S. Alimokhtari[3], K. Black[3] and A. Gow[1]. *[1]EOHSI, Rutgers School of Pharmacy, Piscataway, NJ; [2]EOHSI, Rutgers School of Public Health, Basking Ridge, NJ and [3]EOHSI, Rutgers School of Public Health, Piscataway, NJ.*

Circulating nitrites represent both a marker of endothelial function and an NO source during hypoxia. We have shown that healthy subjects display increased plasma nitrites in response to an ischemic stimulus and that this increase can be abolished by 1-2 hours of traffic related air pollution (TRAP), the same time frame by which TRAP exposure is associated with adverse events such as MI. Dietary inorganic nitrates are reduced to nitrite. This circulating nitrite is reduced in hypoxic environments to NO resulting in increased vasorelaxation. Beets are high in inorganic nitrate. We investigated whether beet juice (BJ) alters the hypoxia response after a TRAP exposure. 20 male and female volunteers participated in a double-blind, randomized, placebo-controlled, crossover study of whether BJ could reduce the oxidant effects of TRAP exposure. Volunteers were randomized to receive 500ml of BJ (20 mM nitrate) or orange juice (OJ), (0.001mM nitrate) on their first visit, and the other on a second visit. Subjects were assessed for blood pressure, exhaled breath condensate (EBC), and for plasma nitrite levels immediately prior and post a 5 min ischemic forearm stimulus. Following EBC and plasma sampling, subjects consumed the juice and then went on a 2 hour rush-hour car ride in the truck lanes of the NJ turnpike. Immediately following each car ride BP, EBC, and plasma nitrite were re-measured. Consumption of BJ significantly increased plasma nitrite (145 nM ± 90.6), while OJ had no effect (-156 nM ± 118.0). For 20 subjects 28 of 38 measurements show an increase in the plasma nitrite in response to ischemia prior to the juice/car ride. After OJ/car ride this fell to 8 of 19, consistent with our previous studies, suggesting oxidative stress. However, following BJ/car ride 10 of 18 post-ride values show a hypoxic plasma nitrite increase, suggesting a protective effect of BJ on endothelial function. EBC data are pending. We propose that the increase in circulating nitrite reduced the endothelial dependence of NO production, and that the oxidative stress induced by TRAP reduced circulating nitrites. These data suggest that beets can supplement circulating nitrites and may represent a nutritional mechanism for supporting endothelial function.

1298 Subacute and Subchronic Inhalation of Aminoethylpiperazine Results in Portal-of-Entry but Not Systemic Effects in Fischer 344 Rats

J. A. Hotchkiss, K. E. Stebbins and B. J. Hughes. *The Dow Chemical Company, Midland, MI.*

Aminoethylpiperazine (AEP; CAS# 140-31-8) is widely used as an intermediate to produce epoxy adhesives, coatings, urethane foams and chelating agents. This study was designed to evaluate portal-of-entry and systemic toxicity resulting from inhalation exposure through epoxy spray applications or spills. F344/DuCrl rats were exposed to 0, 0.5, 11, or 73 mg AEP/m3 for 2 wks or 0, 0.2, 5.2, or 53.3 mg AEP/m3 for 13 wks, and recovery animals were included in all 13 wk exposure groups. The exposure atmosphere was analyzed for both AEP vapor and spontaneously formed AEP-carbamate aerosol (0.9 to 2.0 µm mass median aerodynamic diameter). No treatment-related effects for in-life parameters, hematology or clinical pathology, and no histopathologic evidence of systemic toxicity were observed in rats exposed to any AEP concentration for 2 or 13 wks. Treatment-related histopathology was limited to the anterior nasal cavity and larynx of rats exposed for 2 wks at all AEP concentrations. Histopathologic alterations in rats exposed to 5.2 and 53.3 mg/m3 for 13 wks included atrophy of nasal olfactory, respiratory and transitional epithelium, hyaline droplet formation, epithelial hyperplasia, mucous hypertrophy and chronic active inflammation. Slight-moderate fibrosis and subacute/chronic inflammation in the lamina propria with epithelial hyperplasia and squamous metaplasia in the anterior larynx and slight morphologic alterations to epithelial cells lining the trachea and bronchi were limited to rats exposed to 53.3 mg/m3. A slight increase in absolute/relative lung weight in 53.3 mg/m3-exposed rats was resolved during the 13 wk recovery. The upper respiratory tract is the principal target for inhaled AEP toxicity. No treatment-related alterations were detected in the respiratory tract of rats exposed to 0.2 mg/m3. The no observed effect concentration (NOEC) for systemic toxicity was 73 or 53.3 mg/m3 in rats exposed for 2 or 13 wks, respectively. The subchronic NOEC for portal-of-entry effects was 0.2 mg/m3. Research funded by the REACH Ethyleneamines Consortium

1299 Proinflammatory Responses in Lungs of Rats Exposed to Emissions from 1. and 2. Generation Biodiesel Fuels

P. A. Magnusson[4], J. Gromadzka-Ostrowska[6], O. Myhre[4], H. J. Dahlman[4], J. Biedrzycki[1], M. Gajewska[6], J. Wilczak[6], R. Mruk[6], M. Kruszewski[3], A. Lankoff[3,7] and J. Øvrevik[4]. *[1]Automotive Industry Institute, Warsaw, Poland; [2]Warsaw University of Life Sciences, Warsaw, Poland; [3]Centre for Radiobiology and Biological Dosimetry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland; [4]Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway; [5]Faculty of Human Nutrition and Consumer Science, Warsaw University of Life Sciences, Warsaw, Poland; [6]Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland and [7]Institute of Biology, Jan Kochanowski University, Kielce, Poland.* Sponsor: U. C. Nygaard.

Presently there is limited knowledge on the health impacts of engine emissions from carbon-neutral, renewable fuels such as biodiesel. Diesel engine emissions (DEE) were generated from an Euro 5 engine running on conventional fossil diesel fuel with either 7% or 20% 1. generation biodiesel (B7 and B20), or 20% 2. generation biodiesel (SHB). To assess the efficiency of cleansing technologies, DEE were produced both in presence or absence of a diesel particle filter (DPF). Male Wistar rats were exposed by inhalation to DEE with or without DPF-treatment for 7 or 28 days (6h/day, 5 days/week) in a whole-body exposure chamber. Levels of 29 proinflammatory cytokines in were measured by cytokine-array in bronchealveolar lavage (BAL) fluid. Furthermore, expression of 84 proinflammatory genes was assessed by qPCR-array in lung tissue. BAL fluid of DEE exposed rats contained moderately increased (1.5-3 fold) levels of the chemokines CXCL1/-3/-5/-7, CX3CL1 and CCL20, as well as soluble ICAM-1, L-selectin, TIMP-1 and VEGF. Generally effect appeared to be higher at 7 days compared to 28 days, and most effects appeared to be slightly reduced in the DPF-treated group. In general, there were no marked difference in effects between the different exposure groups expect that B7 induce considerably higher levels of CXCL7 compared to B20 and SHB. No effects of B20 and SHB exposure was observed on gene expression, but B7 induced 2-4 fold increases in a variety of genes both in presence and absence of DPF. Effects on selected cytokines and target genes will now be confirmed by qPCR and ELISA. Increasing the percentage of biodiesel in diesel fuels did not increase the effects of DEE on the investigated markers of pulmonary inflammation. DPF-treatment only moderately affected the results.

1300 Human Equivalent Concentrations for Diacetyl Based on Minimal Bronchiolar Respiratory Effects Observed in Animals: A Refined Analysis Using Current Toxicology Data and Dosimetry Models

M. E. Glynn[2], R. E. Adams[2], E. M. Beckett[2], P. K. Scott[3], J. S. Pierce[2] and B. L. Finley[1]. *[1]Cardno ChemRisk, Brooklyn, NY; [2]Cardno ChemRisk, Chicago, IL and [3]Cardno ChemRisk, Pittsburgh, PA.*

Background: Previous attempts to establish diacetyl exposure-response relationships from flavorings worker data have been confounded by co-exposure to the numerous other respiratory irritants present in flavoring mixtures. In this study, diacetyl human equivalent concentrations (HECs) were derived from bronchiolar effects reported in subchronic rodent inhalation studies. Methods: Benchmark concentration (BMC) modeling was used to estimate diacetyl concentrations associated with a 10% excess risk (BMC10) and the associated 95% lower confidence limits (BMCL10). Additionally, the highest concentrations at which effect incidence and severity were not increased over controls (NOAELs) were determined. HECs were derived from BMCL10 and NOAEL values through the use of dosimetry models and by adjustment for interspecies differences in: 1) scrubbing, 2) lung region surface area, 3) breathing patterns (nose-breathing only and a combination of nose- and mouth-breathing), and 4) experimental vs. occupational exposure durations. Results: For minimal bronchiolar epithelial hyperplasia in rats, the ranges of HECBMCL10 and HECNOAEL values were 1.35 to 33.3 ppm and 1.26 to 27.6 ppm, respectively. The HECBMCL10s and HECNOAELs for minimal peribronchiolar lymphocytic inflammation in mice ranged from 4.26 to 143 ppm and 9.22 to 201 ppm, respectively. Conclusions: These values are significantly higher than, and therefore in conflict with, the HECBMCL10 of 0.60 ppm for diacetyl that NIOSH derived via BMC modeling of abnormal spirometry incidence in a flavoring worker cohort. Animal diacetyl inhalation studies are not subject to the confounding factors experienced by flavoring cohorts and should be considered when evaluating the risk of developing a severe respiratory disorder following diacetyl exposure.

1301 TRPV4 Inhibitor Improves Pulmonary Function and Oxygen Saturation in a Pig Translational Model of Chemically Induced Acute Lung Injury

S. Achanta[1], M. A. Gentile[1], D. J. Behm[2], T. J. Roethke[2], M. D. Gunn[1], I. M. Cheifetz[1] and S. E. Jordt[1]. [1]*Duke University School of Medicine, Durham, NC and* [2]*GlaxoSmithKline Pharmaceuticals, King of Prussia, PA.*

Introduction: The treatment of chemically induced acute lung injury (ALI) remains challenging because of the lack of mechanism-based therapeutic approaches. Chlorine (Cl2) gas is a severe chemical threat with frequent exposures in domestic and occupational environments and in transportation accidents. Cl2 was repeatedly used as a chemical weapon, first in World War I and most recently in Syria, with hundreds of casualties reported there. Our recent studies in a mouse model of Cl2 exposure have shown that transient receptor potential vanilloid 4 (TRPV4), an ion channel expressed in pulmonary tissues, is a crucial mediator of chemically induced ALI. TRPV4 inhibitors ameliorated Cl2 induced ALI in mouse models. In the present study, we asked whether the beneficial therapeutic effects of TRPV4 inhibition would translate to a large animal model of Cl2 exposure, the pig. Methods: Specific pathogen free Yorkshire female pigs (30-40 kg) were mechanically ventilated under anesthesia and exposed to Cl2 gas (≤ 240 ppm for 1 h). A novel TRPV4 inhibitor, GSK691 or vehicle was administered post-Cl2 exposure. Methacholine airway challenge was done. Total and differential leukocyte cell counts in broncho alveolar lavage fluid (BALF) were determined. Lung injury markers were characterized by ELISA and quantitative PCR. Histopathological analysis and scoring was done by a blinded board certified veterinary pathologist. Results: Treatment with GSK691 improved oxygenation and respiratory physiological parameters compared to vehicle group. GSK691 treatment reduced total BALF cell and neutrophil counts. Vascular leakage and edema were decreased in GSK691 treated pigs compared to vehicle group. Pro-inflammatory cytokine markers were decreased and histopathology was improved by GSK691. These results suggest that TRPV4 inhibitors are potential new countermeasures for Cl2 induced ALI; and pave the way for further testing of TRPV4 inhibitor drug candidates following the US FDA's animal rule guidelines (21 CFR 314.600) as an efficacious countermeasure to treat chlorine gas induced ALI in human patients.

1302 Extracted Cookstove Emissions Differentially Alter Pro-Inflammatory and Adaptive Gene Expression in Lung Epithelial Cells

E. A. Gibbs-Flournoy[2], B. Preston[1], M. Hays[4], J. McGee[4], L. Copeland[4] and J. A. Dye[4]. [1]*CSS-Dynamac (Jacobs), Durham, NC;* [2]*ORISE/US EPA, Research Triangle Park, NC;* [3]*NHEERL, US EPA, Research Triangle Park, NC and* [4]*NRMRL, US EPA, Research Triangle Park, NC.*

Current estimates attribute exposure to cookstove emissions (CE) to over 4 million deaths annually. While the development of several new cookstove (CS) designs has led efforts to reduce CE with relative success, the data supporting potential health benefits from the use of new CS remain limited. We previously demonstrated that direct exposure of extracted emissions from three CS: 3-Stone (3S), Natural Draft (ND), and Forced Draft (FD); adversely altered the oxidative status of human (BEAS-2B) and murine (LA-4) lung epithelial cells. Furthermore, *in vivo* exposure of female CD-1 mice substantially influenced lung redox balance. Since CE contain numerous redox-active components including VOCs, PAHs, metals, particulates, and radicals, we hypothesize that oxidative stress is a key mechanistic feature of CE toxicity. As an extension, the current study examined the impact of CE exposure on redox-sensitive inflammatory and adaptive gene expression of both murine and human-derived lung epithelial cell-lines. In brief, LA-4 or BEAS-2B cells were exposed to methanol-derived extracts obtained from filters collected during the aforementioned *in vivo* study. Using real-time quantitative PCR, intracellular responses to each CE extract were examined. The three CE extracts were observed to differentially cause increases in the expression of adaptive, antioxidant, and pro-inflammatory genes including HO-1, GCLC, and IL-8/MIP-2. Like the earlier oxidative stress assessments, gene expression alterations corresponded to the relative emissions of each stove, with the rudimentary 3S stove most often inducing the greatest effect. Together, these data indicate that exposure to various CE has a profound effect on the pro-inflammatory and adaptive gene expression of both human and murine lung epithelial cells. Moreover, the cleaner cooking technologies appear to attenuate the pulmonary epithelial cell toxicity of methanol-extractable emissions. (Does not necessarily reflect US EPA policy.)

1303 Pulmonary Inflammation and Injury Caused by Environmentally Persistent Free Radicals Is Mediated Through Activation of the Aryl Hydrocarbon Receptor

A. C. Harmon[4], V. Y. Hebert[2], S. A. Cormier[3], M. Jennings[4], W. L. Backes[1], R. Reed[1] and T. R. Dugas[4]. [1]*Louisiana State University Health Sciences Center, New Orleans, LA;* [2]*Louisiana State University Health Sciences Center, Shreveport, LA;* [3]*University of Tennessee Health Science Center, Memphis, TN and* [4]*Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, LA.*
Sponsor: T. Dugas.

Combustion of waste at Superfund sites results in the formation of environmentally persistent free radicals (EPFRs), which occur when pollutants are chemisorbed to particles containing redox-active transition metals. Residents near Superfund sites are exposed to these EPFRs through windblown dust, ingestion of soil and sediments, and through emissions occurring from the on-site treatment of contaminants. We have shown that EPFRs produce ROS and when inhaled, lead to pulmonary inflammation, epithelial cell injury and oxidative stress. Prior studies demonstrated that human bronchial epithelial cells (BEAS-2Bs) exposed to EPFRs had increased activation of the aryl hydrocarbon receptor (AhR). However, AhR activation was not reduced by inhibiting cellular uptake, suggesting that AhR activation was induced by an intermediate rather than directly by EPFRs. Thus, our hypothesis was that products of oxidative stress occurring after EPFR exposure stimulate AhR activation, and this AhR activation mediates pulmonary inflammation. To test this hypothesis, BEAS-2Bs were treated with EPFRs and IL-6, TNF-α and ROS were measured in the medium. IL-6, TNF-α and ROS were all increased in the medium of cells exposed to EPFRs versus control cell medium. A subset of cells were also co-treated with an antioxidant or an AhR antagonist, to determine whether oxidative stress and/or AhR activation contributed to an increased cytokine production. EPFR induced AhR activation was dependent upon oxidant production, but ROS production was also somewhat regulated by AhR activation. Preliminary data showed that 8-isoprostane, a lipid oxidation product formed during oxidative stress, is increased in cells treated with EPFRs. Ongoing experiments are aimed at determining whether 8-isoprostane activates the AhR to promote the inflammatory responses observed after EPFR exposure.

1304 Exposure to Inhaled Air Pollutants Results in Altered Barrier Structure in the Duodenal Epithelium of ApoE KO Mice

M. N. Fitch[2], J. Lucero[2], M. J. Campen[3], A. Lund[2] and J. D. McDonald[1]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM;* [2]*Biological Sciences, University of North Texas, Denton, TX and* [3]*College of Pharmacy, University of New Mexico, Albuquerque, NM.*

Recent studies report a global rise in gastrointestinal and metabolic diseases stemming from various ingested environmental toxicants, with the focus primarily on sources such as food and water; however, very few studies have investigated the effects of air pollution on intestinal function and/or diseases to date. The GI tract can be exposed to constituents of air pollution through: ingestion of particulate matter (PM) via mucociliary clearance from the lungs after inhalation, via food and water sources, or systemically through pulmonary translocation. With continued industrialization and increasing subsets of populations being exposed to higher concentrations of air pollution, we investigated whether inhalation exposures to ubiquitous air pollutants, namely wood-smoke and vehicle exhaust, alter duodenal intestinal barrier integrity and structure. Altered intestine barrier function is associated with increased bacterial translocation and resulting systemic inflammation. To investigate the effects of air pollution exposure on the structure/integrity of the gut, 8-10 week old Apo E KO mice, on a high fat diet, were randomly assigned to be exposed to either: wood smoke (WS, ~400 µgPM/m3), mixed vehicular engine emissions (MVE: ~50 µgPM/m3 gasoline engine + 250 µgPM/m3 diesel engine emissions), or filtered air (FA) for a period of 6 hr/day, 7 days/wk for 50 days (n=10 per group). For assessing intestinal structural barrier integrity, we analyzed tight junction (TJ) protein (occludin, claudin-3) and matrix metalloproteinase (MMP)-9 expression using immunofluorescence and confirmed by qRTPCR. MMP-9 has been shown to degrade the epithelial extracellular matrix, while the TJ proteins contribute structurally to the intestinal lining. We observed a significant increase in expression of duodenal MMP-9 with exposure to MVE and WS (~2-fold for each), compared to FA controls. Additionally, we observed a signficiant decrease in expression of duodenal occludin

(~30% reduction in both MVE and WS) and claudin-5 (~10% reduction in WS; 20% in MVE), suggesting increased intestinal permeability in the MVE or WS-exposed mice. These preliminary findings suggest exposure to inhaled air pollutants can alter intestine barrier structure/function. Funded by UNT RIG Grant GA9306 (AKL).

1305 Effects of DEHP and Its Metabolite MEHP on Growth Factors in A549 Cells Are Not Mediated by PPARγ or AhR

L. Rafael-Vázquez and B. Quintanilla-Vega. *Toxicology, CINVESTAV-IPN, Mexico City, Mexico.*

Di(2-ethyl hexyl) phthalate (DEHP) is a common plasticizer used worldwide. Inhalation of DEHP plays an important role in its toxicokinetics. DEHP is metabolized to mono-hexyl phthalate (MEHP), which is considered the most toxic metabolite. Growth factors are implicated in signal transduction, embryonic development, and wound healing, among other effects, and can be modified by toxic compounds. DEHP interacts with peroxisome proliferator-activated receptor gamma (PPARγ) and aryl hydrocarbon receptor (AhR), affecting the transcription of growth factors, cytokines, etc. Our objective was to evaluate the effects of DEHP and MEHP on some growth factors implicated in lung diseases like fibrosis, through the activation of PPARγ or AhR. Pneumocyte-type II cells (A549) were exposed to DEHP (1, 10 and 100 μM) or MEHP (1, 10 and 50 μM) for 24 and 48 h to evaluate the proliferation by the thymidine incorporation assay, and mRNA levels of VEGF (vascular endothelial growth factor), PDGF (platelet derived growth factor), SPRY (protein sprout homolog=activation of fibroblastic growth factor [FGF]), PPARγ and AhR by RT-PCR at 24 h with the co-administration of a PPARγ antagonist (GW9662=2.5 μM) and an AhR partial agonist (α-naphthoflavone=5 mM). We observed a significant proliferative effect at both times with all concentrations of DEHP and MEHP. No significant difference was observed in PPARγ, VEGF or SPRY mRNA with any phthalates, and an increase of AhR mRNA was observed with 100 μM DEHP and a decrease with 1 μM MEHP ($p<0.05$). Interestingly, PDGF mRNA was significantly decreased by DEHP (100 μM), while an increase was observed with MEHP (50 μM). The co-incubation with GW9662 or α-naphthoflavone had no effects, suggesting that DEHP and MEHP act through different mechanisms than the activation of these receptors. Furthermore, our results suggest that DEHP mechanism is independent of MEHP action, and that PDGF modulation could be a mechanism of DEHP toxicity to explain its effects on lung development and may participate in the induction of some diseases as fibrosis.

1306 Inflammatory Responses and Its Mechanisms Induced by Polyhexamethylene Biguanide

H. R. Kim, J. J. Kim, D. Y. Shin and K. H. Chung. *Sungkyunkwan University, Suwon, Korea, Republic of.*

Polyhexamethylene biguanide (PHMB) is one of polymeric guanidine family, which is used as antimicrobial agent to sterilize swimming pool, contact lens and so on. Some studies reported that polyhexamethylene guanidine phosphate, which is another member of guanidine family, induced massive inflammation and fibrosis in lungs. However, inflammatory effects of PHMB in lung are unclear. The aim of this study is to elucidate the inflammatory responses and its mechanisms induced by PHMB in lung cells. Firstly, WST-1 assay was conducted to find appropriate exposure concentration. Reactive oxygen species (ROS) generation was measured by DCF-DA assay. In addition, ELISA assay was performed to measure release of inflammatory cytokines such as TNF-a, IL-6 and IL-8. NF-κB activation was identified by western blot. Furthermore, various inhibitors were used to elucidate the mechanisms in PHMB-induced inflammation. Dose-dependent cytotoxic effects were observed in A549 cells. PHMB slightly increased ROS and TNF-α and triggered the activation of NF-κB by modulating the degradation of IκB-α in A549 cells. In addition, PHMB up-regulated IL-6 and IL-8, which were prevented by inhibitors.

1307 Chronic Over-Consumption of Alcohol Reduces Levels of Antimicrobial Peptides (LL-37) in Human Broncho Alveolar Lavage Fluid (BALF), Pulmonary Monocytes and Alveolar Epithelial Cells

O. Ogunsakin, H. Hottor and M. McCaskill. *School of Public Health, Tulane University, New Orleans, LA.*

Introduction: Antimicrobial peptides like LL-37 are activated and up-regulated by Vitamin D in the pulmonary system. These peptides help in fighting pathogens, thus indirectly preventing the onset of infection among healthy humans. Chronic over consumption of alcohol, however, has a deleterious effect on the pulmonary system by significantly depleting levels of active vitamin D, which indirectly results in down-regulation of anti-microbial peptides, thereby making vulnerable subjects prone to severe respiratory infection. Methods: THP-1 human acute monocytic cell lines were pre-treated with 80 mM alcohol for 24 hours, and treated with 1 μM of DADS for 6 and 24 hours. Human Pulmonary Alveolar Epithelial cells (hPAE) were also cultured in recommended media, AEPiCM, then treated with ethanol and DADS as in the THp-1 cells. Bronchioalveolar Lavage Fluid (BALF) samples of subject with Alcohol Use Disorder (AUD) were obtained from COPARC databank and exposed to the same alcohol and DADS treatment. Following treatment, the cells were lysed and the homogenate was prepared for assay. Levels of LL-37 protein were quantified in all these cells and samples using Human Cathelicidin/LL-37 (Hycult Biotech) assay kits. Results: THP-1 cells exposed to Alcohol (80 mM) displayed approximately 54% reduction in the cellular concentration of LL-37 proteins. This reduction was reversed by treatment with DADS for a 6 hour duration. LL-37 levels were statistically reduced by 50% in the hPAE cells treated with ethanol. The LL-37 levels were reversed back to 70% of control upon treatment with DADS. Additionally the BALF samples showed 25% reduction in the concentration of LL-37 proteins when exposed to alcohol. The levels of LL-37 in the cell samples were statistically reduced ($p<0.05$) by ethanol, but reversed back by treatment with DADS concentrations. Conclusions: Our study showed that chronic over-consumption of alcohol has the potential to inhibit the ability of THP-1 cells to activate antimicrobial peptide, LL-37, by depleting the levels of pulmonary vitamin D. In addition, our study demonstrated that DADS has the ability to attenuate alcohol-induced depletion of active vitamin D and down-regulation of LL-37 in the BALF samples, THP-1 and hPAE cells.

1308 39-Week Inhalation Toxicity and Fibrogenicity of Abrasive Blasting Sand and Specular Hematite

W. M. Gwinn[2], M. F. Cesta[2], M. D. Stout[2], J. H. Roycroft[2], C. S. Smith[2], J. A. Dill[1], G. L. Baker[1], A. Gupta[1], B. K. Hayden[1], K. M. Patton[1] and D. Germolec[2]. [1]*Battelle, Richland, WA and* [2]*DNTP, NIEHS, Research Triangle Park, NC.*

Blasting sand (BS), most commonly used in abrasive sandblasting, contains high levels of crystalline silica and thus has the potential to cause pulmonary fibrosis (silicosis) following inhalation exposure. Alternatives to BS exist with lower crystalline silica content, including specular hematite (SH), but no comprehensive inhalation studies have been performed to evaluate the health effects (pulmonary toxicity). Therefore, inhalation toxicity studies were performed with BS (>75% crystalline silica) or SH (>95% iron oxide, 1-2% crystalline silica). Pulmonary toxicity, fibrogenicity, immunotoxicity and tissue burden were compared in HSD rats after whole body exposure to target aerosol concentrations of 0, 15, 30 or 60 mg/m3 BS or SH for up to 39 weeks. There were no treatment-related changes in survival, body weights or clinical observations for either compound. There was a significant dose-dependent increase in lung and bronchial lymph node weights for both compounds compared to controls. Except for 15 mg/m3 SH, lung overload was achieved by week 39 for both compounds at all concentrations. There was a significant dose-dependent increase in total cells (alveolar macrophages and PMNs), LDH activity and MCP-1 protein levels in BAL fluid compared to controls for both BS and SH. In the nose, respiratory epithelial hyaline droplet accumulation was less apparent in SH-exposed rats compared to BS. In the larynx, SH but not BS induced squamous metaplasia of the epiglottis. Lung histopathology showed that the incidence of chronic active inflammation and interstitial fibrosis were both less in SH-exposed rats compared to BS at week 26 for all concentrations and at week 39 for 15 mg/m3 SH. In addition, alveolar proteinosis was observed in the lungs of rats exposed to 60 mg/m3 BS but not SH. These data suggest that SH as an alternative abrasive blasting agent to BS may be less toxic and fibrogenic to the lower respiratory tract likely due to lower crystalline silica content.

1309 Circulating Inflammatory Potential in Obstructive Sleep Apnea Patients

K. Zychowski[3], B. Sanchez[3], G. Herbert[3], R. P. Pedrosa[2], G. Lorenzi-Filho[2], L. Drager[2], V. Y. Polotsky[1] and M. Campen[3]. *[1]Johns Hopkins University School of Medicine, Baltimore, MD; [2]University of São Paulo Medical School, São Paulo, Brazil and [3]Department of Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

Introduction: Obstructive Sleep Apnea (OSA) is a serious disorder affecting 22 million Americans. OSA is characterized by repeated episodes of hypoxia and arousal, resulting in detrimental systemic physiological changes, including vasoconstriction, vascular remodeling and ultimately cardiovascular disease. Numerous studies have found that inflammatory markers and endogenous toxins are elevated in the circulation of OSA patients, which may explain the numerous associated comorbidities. Methods: Age and body mass index matched cohorts of healthy (n=7) and OSA subjects (n=16) were recruited. OSA subjects were randomized to receive CPAP (n=8) or no treatment therapy (n=8) for three months. All subjects were male, ages 32-55. To test the overall inflammatory potential of the serum from these subjects, we used a novel endothelial biosensing assay. Human coronary artery endothelial cells (hCAECs) were incubated with 5% serum from individual subjects for 4h and qPCR was performed to assess inflammatory transcripts (ICAM-1, VCAM-1 and IL-8). Results: Circulating inflammatory potential, in terms of endothelial VCAM-1, ICAM-1 and IL-8 mRNA response to serum, was significantly increased in OSA subjects compared to controls. Additionally, there were decreasing trends in ICAM-1 and VCAM-1 mRNA resulting from CPAP therapy, opposite of the sham condition. Serum concentrations of C-reactive protein were elevated in OSA patients, but sVCAM, sICAM and serum amyloid A were similar between groups. Conclusions: CPAP may reduce circulating proinflammatory factors contributing to endothelial cell activation. Further molecular studies are needed to elucidate the specific mechanisms responsible for this effect and the relationship to clinical cardiovascular outcomes.

1310 Threshold for the Interactive Effects of Cigarette Smoke and Asbestos Exposure for the Risk of Lung Cancer

E. S. Fung, M. Kovochich, D. M. Cowan and A. K. Madl. *Cardno ChemRisk, LLC, Aliso Viejo, CA.*

Cigarette smoke is the leading cause of lung cancer. Exposure to cigarette smoke and asbestos is one of the most widely cited examples of a synergistic response of mixtures. However, "synergy" or interactive effects of smoking and asbestos for the risk of lung cancer is often not considered in the context of dose, asbestos fiber type, presence/absence of asbestosis, and influence of confounding risks. An evaluation of the existing epidemiologic data was undertaken to identify a threshold dose at which asbestos exposure may significantly add to the risk of lung cancer from smoking alone, as well as evaluate the influence of fiber type and dose on the strength of the interaction between smoking and asbestos for the lung cancer risk. An inclusion-exclusion criteria model was used to account for confounding variables and to stratify lung cancer risk data into categories that: 1) compared smoking alone versus smoking and asbestos, 2) adjusted for smoking, 3) included but did not analyze data from category 1, 4) compared asbestos versus smoking and asbestos, 5) lacked asbestos only or smoking only controls, or 6) did not adequately study the effects of smoking. Although the majority of epidemiologic studies have significant confounding factors, a subset of data provide clarity on the threshold for the interactive effects of smoking and asbestos compared with the risk of lung cancer from smoking alone. It was observed that asbestos doses at or below 25 f/cc-yrs do not additionally increase the risk of lung cancer from smoking. High exposures to asbestos (particularly amphiboles), cigarette smoke (≥40 pack-yrs), and the presence of fibrotic lung disease was associated with a more than additive interaction for the risk of lung cancer. Our analysis indicates that any potential "synergistic" interaction between smoking and asbestos occurs at high asbestos exposures and in the presence of fibrosis and there is no interaction at doses below 25 f/cc-yrs for asbestos.

1311 Identifying Genetic Predictors of Response to Respiratory Toxicants Using the Collaborative Cross Mouse Genetic Reference Population

S. Kelada. *Genetics, University of North Carolina, Chapel Hill, NC.*

Identifying the genetic determinants of variation in response to toxicants provides insight into sources of inter-individual variation and toxicologic mechanisms. However, identifying such gene-environment interactions is a difficult task in epidemiologic studies due to a number of vexing methodological and statistical issues, thus complementary approaches are clearly needed. We aim to identify genetic loci that control response to respiratory toxicants using a new mammalian genetic reference population, the Collaborative Cross (CC). The CC is a panel of recombinant inbred strains of mice derived from eight-way crosses using five classical inbred strains (C57BL/6J, 129S1/SvImJ, A/J, NOD/ShiLtJ, and NZO/H1LtJ) and three wild-derived inbred strains (WSB/EiJ, PWK/PhJ, and CAST/EiJ). The abundant genetic variation present in CC lines makes this a unique and powerful tool for detection of gene-environment interactions. As a proof of concept, we employed a house dust mite model of asthma which produces hallmark disease phenotypes including airway inflammation, mucin hyper-secretion and airway hyper-reactivity. We phenotyped 150 nascent CC lines for airway inflammation (total and differential cell counts in lung lavage fluid), mucin protein levels, and also measured gene expression in whole lung RNA using microarrays. After high density, genome-wide genotyping, we identified genetic loci that control inflammation phenotypes and gene expression using quantitative trait locus (QTL) mapping. We identified a locus on chromosome 11 that regulates eosinophil recruitment to the airways. For neutrophil counts, we identified loci on chromosomes 2, 4 and 7, and we used gene expression data to identify a candidate gene for the chromosome 7 locus, namely Zfp30. Loci that regulate concentrations of the mucins MUC5AC and MUC5B were found on chromosomes 13 and 2, respectively. Using additional bioinformatic tools and resources, we identified a small number of promising candidate genes for each mucin locus. These results demonstrate that the CC is a promising resource that can be used to identify the genetic underpinnings of responses to respiratory toxicants and set the stage for further studies with additional toxicants.

1312 Altered Metabolism of Lung Mesothelial Cells in Response to Asbestos Exposure

C. Mesaros[3], D. Ciccimaro[3], L. Guo[3], N. W. Snyder[1] and I. A. Blair[3]. *[1]A.J. Drexel Autism Institute, Drexel University, Philadelphia, PA; [2]Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA and [3]Department of Systems Pharmacology & Translational Therapeutics, University of Pennsylvania, Philadelphia, PA; [4]Penn SRP Center, University of Pennsylvania, Philadelphia, PA.* Sponsor: T. Penning.

Asbestos exposure is a pervasive occupational health hazard as well as a major public health concern. In order to better monitor subjects who are at risk for developing asbestos-related diseases prior to disease onset, it is necessary to identify cellular biomarkers of asbestos exposure that are mechanistically relevant to the disease pathogenesis, which results in mesothelioma. Mesothelial cells form a monolayer of specialized pavement-like cells that line the body's serous cavities and internal organs including pleura (thoracic cavity), peritoneum (abdominal cavity including the mesentery) and pericardium (heart sac). The primary function of this layer, termed the mesothelium, is to provide a slippery, non-adhesive and protective surface. Currently, the mechanisms by which asbestos induces damage to mesothelial remain unclear. However, a large body of evidence supports the hypothesis that asbestos increases the cellular oxidative stress. In the context of chronic asbestos exposure, this oxidative stress is thought to induce tissue damage and inflammation, which can contribute to carcinogenesis. Since biomarkers of oxidative stress are not specific to asbestos exposure, we used a cell culture models to examine the effects of asbestos on mitochondrial metabolism may lead to the elucidation of biomarkers exclusive to asbestos exposure. Lung mesothelial cells were exposed to different types of asbestos for up to two weeks. Using liquid chromatography mass spectroscopy (LC-MS) methods we monitored how asbestos exposure altered the levels of Krebs cycle metabolites, glycolysis and fatty acids synthesis. Mesothelial cells were incubated with, [13C6]-glucose, [13C515N1]-glutamate, or [13C16]-palmitate as described recently for neuronal cells (Worth et al. J Biol Chem. 2104;289:2689) in order to determine which pathways of metabolism were disrupted by asbestos exposure. Our results will contribute to the characterization of biomarkers that are unique to asbestos exposure thereby facilitating intervention prior to disease onset.

1313 Toxicological Effects of Inhaled Fracking Sand Dust on Reactivity and Neurogenic Responses of Isolated Rat Trachea

K. A. Russ, W. McKinney, A. M. Cumpston, M. C. Jackson, J. Thompson and J. S. Fedan. *National Institute for Occupational Safety and Health, Morgantown, WV*. Sponsor: J. Roberts.

Hydraulic fracturing (fracking) entails pumping a fluid and sand (silica) mixture through drilled wells to cause the fracturing of shale deep underground, resulting in the release of natural gas. During this process, workers at fracking sites are exposed to large amounts of respirable sand dust as it is prepared for pumping into the well. Inhalation exposure to high levels of crystalline silica is known to produce silicosis. To investigate the toxicological effects of inhaled fracking dust, rats were exposed to fracking sand dust (collected at a fracking site) at 10 mg/m3 or 30 mg/m3 for 6 hours per day for 4 consecutive days. Tracheas were isolated from euthanized animals for *in vitro* preparations. In the isolated, perfused trachea at day 7 post-exposure to 10 mg/m3 the reactivity to methacholine (MCh) applied to the extraluminal surface of the trachea was increased as compared to air-breathing control animals. Contractile responses to intraluminally-applied MCh were unaffected by dust inhalation at any of the post-exposure time points examined. In tracheal strips, electrical field stimulation evoked contractions that were not affected by dust inhalation of 10 mg/m3 or 30 mg/m3 at any of the post-exposure time points. These findings suggest that at 10 mg/m3, reactivity of airway smooth muscle to methacholine was increased but effector nerve function was not altered at either 10 or 30 mg/m3. No changes to the integrity of the epithelial cell lining of the trachea were observed.

1314a Role of Osteopontin & Proteinase 3 in BAL & Blood in Phosgene-Induced Lung Edema

W.-L. Li[1] and J. Pauluhn[2]. [1]*Department of Toxicology, 4th Military Medical University, Xi'an, Shaanxi, China and* [2]*Global Phosgene Steering Group, Bayer MaterialScience AG, Leverkusen, Germany*.

The intervention principles of phosgene-induced pulmonary edema are controversially discussed and commonly countermeasures focus on anti-inflammatory principles. One of the key mechanism of phosgene is to deplete glutathione (GSH) in the lining fluids of the lung and the function of alveolar macrophages (AM) is increasingly compromised due to the limited availability of GSH. Recent evidence suggests that any reduction of the redistribution of vascular fluid from the systemic circulation into the lung prevents edema occurrence and progression if administered early enough postexposure. This study examined rats exposed to 1000 mg/m^3 x min phosgene gas. Air exposed rats served as control. All animals were examined on the climax of edema occurrence 1-day post-exposure. Leukocytes (neutrophilic granulocytes, PMN), osteopontin (OPN) and PMN-specific proteinase (PR3) were analyzed in blood. Red blood cells (RBC) counts and hematocrit were used to estimate any degree of hemoconcentration. These endpoints were compared with PMN-counts, OPN- and PR3-protein in bronchoalveolar lavage (BAL)-fluid and BAL-cells. Total protein and collagen in BAL-fluid served as endpoints mirroring the magnitude of edema formation. Hemoconcentration coincided with significantly decreased PMN counts and PR3 in blood. This was contrasted by increased BAL-PMN counts and PR3 in BAL-cells. AM were decreased by 50% and 1/3 of them had a foamy appearance. OPN in BAL-cells was minimal relative to BAL-fluid. The most salient increase of OPN occurred in plasma. In summary, the extravasation of collagen into the alveolus paralleled that of total protein. The changes of PR3 appear to mirror the counts of PMN in the respective compartment. Its moderate increase supports the conclusion that PMN migrate into the lung concomitant with extravasated proteins in the absence of any appreciable activation. AM appear to be particularly susceptible to an almost lethal exposure dose of phosgene. More research seems to be warranted on early countermeasures focusing on maintaining the functional integrity of the AM to minimize the generation of metabolites affecting cardiopulmonary function rather than modulating any PMN-related inflammatory responses.

1314 Instillation of Indium-Tin Oxide Production Facility Particles in Rats Induces Pulmonary Toxicity

M. Badding[4], N. Fix[2], M. Orandle[1], M. Barger[1], K. Dunnick[3], K. Cummings[1] and S. Leonard[1]. [1]*National Institute for Occupational Safety and Health, Morgantown, WV;* [2]*State University of New York at Buffalo, Buffalo, NY;* [3]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [4]*Center for Toxicology and Mechanistic Biology, Exponent, Alexandria, VA*.

Indium-tin oxide (ITO) is a mixture of metal oxides and is used to make transparent conductive coatings for touch-screen and liquid crystal display electronics. As the demand for consumer electronics continues to rise, concern over occupational exposures to potentially toxic particles generated during ITO production has increased. Epidemiologic studies have shown an association between workers exposed to ITO and development of pulmonary alveolar proteinosis (PAP) and fibrotic interstitial lung disease. Our previous *in vitro* studies have demonstrated cytotoxicity and inflammasome activation in response to various indium-containing particles. However, which of these indium materials or specific industrial processes may be the most toxic *in vivo* remains unknown. In the current study, particle samples were collected at different production stages throughout an ITO facility and various endpoints were evaluated over a time course following rat pulmonary exposures to these particles. Indium oxide (In2O3) is a starting material, sintered ITO (SITO) is generated during the grinding of the final ITO product, and ventilation dust (VD) is present during reclamation of indium from leftover materials (and therefore, contains SITO). Rats were exposed via a single intratracheal instillation dose of 0 mg (PBS vehicle control), 0.5 mg (VD only), 1 mg, or 5 mg per rat (n = 6-8/group). Each particle type induced pulmonary inflammation and damage in rats (evaluated at 1, 7, and 90 days post-instillation), but SITO and VD caused the most damage when responses at the same dose were compared on days 7 and 90. SITO and VD also led to significantly higher plasma indium levels than In2O3. Downstream pathological changes such as PAP and fibrosis were observed in response to all three particles 90 days post-treatment, with a trend towards greatest severity in animals exposed to VD when comparing animals that received the same dose. These findings may inform workplace exposure reduction efforts and provide a better understanding of the pathogenesis of this emerging occupational health issue.

1315 A Comparative Study of Essential Oils and Their Chemotypes on A549 Lung Cancer Cells

E. R. Esposito[2], K. Adair[2], K. Murphy[2], S. Baltzley[2] and A. AlGhananeem[2]. [1]*Sullivan University College of Pharmacy, Louisville, KY and* [2]*Pharmaceutical Sciences, Sullivan University College of Pharmacy, Louisville, KY*.

Purpose: Essential oils (EOs) are concentrated from plants and carry a distinctive "essence" that is not limited to perfumery. Essential oils have been used for healing properties and may provide an adjuvant method for treating diseases such as lung cancer. While some studies examine constituents of specific compounds on various cancer cells lines, current literature is limited on chemotype apoptotic effects. A chemotype is a chemically distinct entity within a plant species; Thymus vulgaris has 7 possible chemotypes, each of which will change the properties of the EO if it is the dominant component. We hypothesize that EOs (frankincense, oregano, nutmeg, myrrh) and thyme chemotypes (Thyme thujanol, Thyme linalool, Thyme thymol) will induce apoptosis in lung (A549) cancer cell lines. The objective of this study was to determine if thyme EOs containing different chemotypes have altered apoptotic activity in A549 cells. Methods: Cells were cultured and maintained using standard ATCC protocols. Cell viability was determined by Alamar Blue assays. Cells were plated in 96-well plates and serial dilutions of EOs/chemotypes were added for 12 hours with either 1:250, 1:500, 1:750, or 1:1000 dilutions of EOs and compared to control. Intracellular caspase detection was assessed at 24 hours using the same methodology. Based on results chemotypes were screened using HPLC for constituent activity. Results: Data suggests oregano and the thyme chemotypes exhibited the best cytotoxic activity against A549 cells. Viability decreased by 20% for oregano at concentration ratios of 1:500 and 1:750; Thyme linalool at 1:250; and Thyme thymol at 1:500, 1:750, and 1:1000. Caspase activation was observed in Thyme linalool, and Thyme thymol chemotypes. HPLC data confirmed that active chemotypes (Thyme linalool, and Thyme thymol) contained a distinct peak for thymol, while Thyme thujanol did not contain thymol. Data are the average of three independent experiments ± (SEM), $p < 0.05$. Conclusions: Essentials oil chemotypes containing thymol exhibited the greatest decrease in cell viability and demonstrated caspase activation. However, not all chemotypes within the same plant species contain the same constituents. When considering EO use, plant chemotypes may play an important role in determining cancer cell viability.

1316 p53-Independent Activation of p21 in Response to Mitomycin C and Decarbamoylmitomycin C

J. Seo, B. T. Huang, S.-Y. Cheng and E. Champeil. *Sciences, John Jay College of Criminal Justice, New York, NY.*

Mitomycin C (MC) has been used to treat cancer. Experimental evidence points to crosslinks, α-ICL and β-ICL (interstrand cross link), as being the lesions primarily responsible for the cytotoxicity of the mitomycins. 10-decarbamoyl mitomycin C (DMC), an analog of MC, can form a similar ICL with DNA as MC (α-ICL). DMC additionally generates a major unique stereoisomeric ICL (beta isomer, β-ICL). Studies found that DMC was more toxic in cancer cells without functioning p53 as compared with MC. MC and DMC differ in their signaling responses. Literature shows that the p21 pathway is important in MC and DMC biochemistry. Our hypothesis is that MC and DMC provoke a p21 cell death in a p53 independent manner. MCF-7 (p53 proficient cells) and K562 (p53 deficient cells) were used for this study. Cells were treated with various concentrations of MC and DMC for 24 hours. After chemical exposures, cells were subjected to cell cycle study and Western blot analysis to determine the activation of p21. Short hairpin RNA for p53 was used to knockdown p53 expression in MCF-7 to evaluate the role of p53 in the activation of p21 by MC and DMC. Cell cycle results show DMC induced cell cycle arrest at G1/G0 for MCF-7 and a shift to S and G2/M for K562. MC induced cell cycle arrest at G1/G0 for MCF-7 but only induced a shift toward S phase for K562. DMC shows clear and consistent trend while MC shows more irregular trend, especially at low concentrations for MCF-7. MC significantly triggered the de-phosphorylation of p21 at Thr145 after 24 hours exposure to 50 μM MC on both MCF-7 and K562. At the highest tested dose, there was reduction in de-phosphorylation but the effect is not significant in either cell line. DMC significantly reduced the level of p21 phosphorylation at Thr145 after 24 hours exposure to 50 μM for MCF-7 cells and to 75 μM for both MCF-7 and K562 cell lines. There was a trend of reduction observed in MCF-7 cells treated as low as 5 μM DMC. With shRNA p53 knockdown, the activation of p21 by MC and DMC was not attenuated. This indicates the activation of p21 by MC and DMC is p53-independent.

1317 Gastric Mucosal Cells as an Early Screening Assay for Drug Toxicity

S. Goineau. *Porsolt Sas, Le Genest Saint Isle, France.* Sponsor: G. Froget.

Gastric mucosa is frequently exposed to various gastric irritants including drugs, alcohol, and hydrochloric acid. Primary cultures of gastric mucosal cells offer a promising tool for evaluating the cytotoxic or protective effects of compounds, in the absence of systemic factors which can complicate the interpretation of *in vivo* data. However, data from animal models are still essential to address the clinical question. Therefore, preclinical studies conducted in both *in vitro* and *in vivo* rat models may provide a more comprehensive insight into the effects of compounds on the gastric mucosa. Gastric mucosal cells were isolated from rat stomach fundus. Cells (5x104 cells/ml in 24-well plates) were seeded and cultured at 37°C in a 5% CO2 / 95% air incubator. Following incubation with vehicle, test or reagent substances, the cell culture media were collected for lactate dehydrogenase release measurement (indicator of plasma membrane failure). The gastric mucosal cells were then incubated with a MTT solution and cell viability was assessed 4 hours later (quantitative colorimetric measurement of mitochondrial activity). In *in vivo* studies, rats were administered with vehicle or test substance before an acute dose of indomethacin. Six hours later, the stomach was removed and opened to macroscopically examine gastric lesions. *In vitro*, indomethacin (1 to 10 mM) induced, in a concentration-dependent manner, gastric cell injury from 3 mM. Rebamipide (0.3 and 1 mM) and sucralfate (3 mg/ml) reduced the indomethacin-induced cell damage. *In vivo*, indomethacin (30 mg/kg, p.o.) caused the occurrence of gastric ulcers 6 hours post-dosing. Sucralfate (300 mg/kg, p.o. administered 1 hour before indomethacin) exhibited gastroprotective effects against indomethacin-induced gastric lesions (decrease in the length of the gastric lesions and reduction in number of ulcers). These findings suggest that, in addition to standard *in vivo* gastrointestinal assays, cultured gastric mucosal cells can provide a suitable model for studying the effects of drugs on gastric epithelial cell functions. These primary cell models could therefore be proposed for short-term toxicity studies and early drug screening (cell viability and cytotoxicity assays).

1318 Crizotinib and Ceritinib Induce Apoptosis and Necrosis in Primary Rat Hepatocytes at Therapeutic Levels

Q. Shi, X. Yang, J. J. Greenhaw and W. B. Mattes. *Division of Systems Biology, US Food and Drug Administration (US FDA), The National Center for Toxicological Research (NCTR), Jefferson, AR.*

Crizotinib and ceritinib are two anaplastic lymphoma kinase (ALK) inhibitors recently approved by the FDA for treating metastatic non-small cell lung cancer (NSCLC) patients with ALT abnormality. Clinical trial data show that both drugs caused liver injury, and this information has been included in the "Warnings and Precautions" section of the labeling. Given that the mechanism of hepatotoxicity is unknown, this study was undertaken to determine if crizotinib and ceritinib are directly toxic to liver cells. Primary cultured rat hepatocytes were treated with crizotinib and ceritinib at clinically relevant concentrations for 4, 8 and 24 h, and apoptosis and necrosis were measured. A 2 to 5-fold increase in caspase 3/7 activity was observed at 8 and 24 h, and significant necrosis (~80%) occurred at 24 h. Significant cytochrome c release was observed at 4 h, the time point when no cell death occurred. Apoptosis was also confirmed by nuclear fragmentation and condensation, and caspase inhibitors partially abrogated cell death. These changes were triggered by ceritinib at 3 to 6-fold the maximal blood concentration (Cmax) and by crizotinib at 15 to 30-fold Cmax. Crizotinib showed no overt toxicity at 10-fold Cmax, while ceritinib became toxic at 3-fold Cmax and caused 100 % cell death at 6-fold Cmax, indicating that ceritinib, the second-generation ALK inhibitor, is significantly more cytotoxic than the first-generation drug crizotinib. These data provide novel insights into the mechanisms of crizotinib and ceritinib associated hepatotoxicity.

1319 Comparative Cytotoxicity of Halobenzoquinone Isomers in Mammalian Cell Lines

J. Li, S. Vemula and X.-F. Li. *University of Alberta, Edmonton, AB, Canada.*

Halobenzoquinones (HBQs), a new class of drinking water disinfection byproducts (DBPs), are cytotoxic to human bladder cells T24 and Chinese hamster ovary cells (CHO). However, the isomeric effects of HBQ-cytotoxicity are unknown. This study is to examine isomeric structure effects on cytotoxicity. Specifically, I will study cytotoxic potency of two pairs of HBQ isomers, including 2,6-dicholoro-1,4-benzoquinone (2,6-DCBQ) and its isomer 2,5-dicholoro-1,4-benzoquinone (2,5-DCBQ), 2,6-dibromo-1,4-benzoquinone (2,6-DBBQ) and its isomer 2,5-dibromo-1,4-benzoquinone (2,5-DBBQ). The cytotoxic effects of HBQ isomers on both T24 and CHO cells were first determined using an impedance-based real-time cell analysis (RTCA) method. In parallel, cellular reactive oxygen species (ROS) induced by HBQs was measured. The RTCA monitors the real-time response of cells to these isomers and generates half-inhibitory concentration (IC50) for each isomer. The results show that the cytotoxic effects of HBQ isomers are in a concentration- and time- dependent manner in both cell lines. The rank order of cytotoxicity of HBQ isomers was 2,5-DBBQ > 2,5-DCBQ > 2,6-DBBQ > 2,6-DCBQ in CHO cells, and was 2,5-DBBQ > 2,6-DBBQ > 2,5-DCBQ > 2,6-DCBQ in T24 cells. Production of ROS was also concentration dependent, and 2,5-HBQ isomers produce higher ROS levels than their 2,6-HBQ isomers. Furthermore, HBQ isomers cytotoxicity is correlated with ROS formation, supporting oxidative pathway involved in cytotoxicity. In conclusion, 2,5-isomers of HBQs are more toxic than the corresponding 2,6-isomers, and ROS formation correlated with their cytotoxicity.

1320 The Curcumin Derivative RL112 Induces Apoptosis in Ovarian Cancer Cell Lines

P. Gwavava, M. Nimick, S. Taurin, B. C. Hawkins and R. J. Rosengren. *University of Otago, Dunedin, New Zealand.*

Ovarian cancer is the most lethal gynecological cancer in women and thus new safe and effective drug therapiers are needed. We have previously shown that second generation curcumin derivatives are selectively cytotoxic to cancer cells of the breast and prostate, with many derivatives exhibiting EC50 values below 1 μM. In the search for new drugs for ovaraian cancer we examined the efficacy of 14 different derivatives in SKOV-3 and OVCAR-3 cells. Cells were treated with 0 - 10 μM of the derivatives for 72 h. Cell number was determined with the sulforhodamine B assay and EC50 curves were generated using Graphpad Prism software. 1-methyl-3,5-bis(3-nitrobenzylidene)-4-piperidone (RL112) was the most potent derivative with EC50 values of 0.52 μM and 0.64 μM in SKOV3 and OVCAR3 cells, respectively. 3,5-bis (3,4,5-trimethoxybenzylidene)-1-methylpiperidine - 4 - one (RL71) and 1- isopropylyl- 3, 5- bis (3,4 methylene-dioxyl- benzylidene) - 4 - piperidone (RL117) also elicited submicromolar EC50 values. Flow cytometry was

then performed to determine the effect of RL112 on cell cycle progression and apoptosis. Treatment with RL112 (0.75 μM) increased G2/M-phase arrest in SKOV-3 cells by 89 and 153% compared to control following 48 and 72 h, respectively. However, there was no change in cell cycle progression in OVCAR-3 cells. Inductin of apoptosis in SOV3 cells was time and concentration-dependent. Specifically, apoptosis was significantly increased from control at 24 h and by 72 h 25% of the cell population was apoptotic. Western blotting of key proteins in that drive cell proliferation showed that RL112 reduced the expression of NF-κB by 72%. Overall the results have shown that the curcumin derivative RL112 is a lead compound in the development of drugs for ovarian cancer and warrants further investigation in animal models.

1321 Celastrol Induced Cytotoxicity and Apoptosis in A549 Cells

B. Alpertunga and T. Jannuzzi. *Department of Pharm Toxicolo, Istanbul University Faculty of Pharmacy, Istanbul, Turkey.*

Celastrol is an important bioactive compound extracted from Triptergium wilfordii Hook F. This medicinal plant widely and successfully used in the treatment of inflammatory disease in China. So far, celastrol was shown to have beneficial effects *in vitro* and *in vivo* on various types of cancers. However study of celastrol-induced apoptosis on non-small-cell lung cancer A549 cells is limited. MTT assay was used to evaluate the cytotoxicity of the cells following treatment. Our results showed that celastrol inhibits the growth and proliferation in a concentration-dependent manner of A549 cells with an IC(50) of 2.02 μM at 24 h treatment. By using Annexin V/Propidium iodide fluorescent staining based cells apoptosis analysis, it has been shown that main mechanism of cytotoxic effects of celastrol in A549 cells were through apoptosis induction. Following 24 h celastrol treatment with the concentrations of 0.5, 1, 2, 3 and 4 μM, the rate of apoptosis compared to control cells (2.06%) was increased to 6.46% 12.22%, 14.47%, 17.63% and 21.65%, respectively. Our results indicated that celastrol may inhibit the cell proliferation and induce apoptosis of human non-small-cell lung cancer A549 cells. As a conclusion, further studies are needed to consider celastrol's potential role in cancer chemotherapy.

1322 Methylmercury Induces Oxidative Stress, Caspase-3 Activation and Cleavage of Rock-1 in Primary Astroglial Cells

A. Antunes dos Santos and M. Aschner. *Albert Einstein College of Medicine, New York, NY.*

Methylmercury (MeHg) is a highly toxic environmental contaminant that produces neurological and developmental impairments in animals and humans. Although its neurotoxic properties have been widely reported, the molecular mechanisms by which MeHg exerts its toxic effects are not completely understood. The Rho-associated protein kinase 1 (ROCK-1), is a key regulator of actin cytoskeleton and a direct substrate of caspase-3. The activation of ROCK-1 is necessary for membrane blebbing during apoptosis. The present study investigated the hypothesis that the oxidative stress induced by MeHg in astrocytes triggers the activation of the caspase-dependent apoptotic pathway, leading to the cleavage and activation of ROCK-1. Primary astrocytes were isolated from cortical tissue of postnatal day-1 C57BL/6 mice. After 2 weeks in culture, the astrocytes were treated with 10 μM MeHg for 6 h, a concentration that did not significantly alter cell viability. Two fluorescent dyes were used to measure the intracellular and mitochondrial reactive oxygen species formation (CM-H2 DCFDA and CM-H2 XRos, respectively). To test the hypothesis that MeHg induces ROCK-1 cleavage in astrocytes, we carried out western blotting analysis of the active forms of caspase-9, caspase-3 and ROCK-1. Our results establish that astrocytes treated with 10 μM MeHg for 6 h showed a significant increase in the intracellular and mitochondrial reactive oxygen species levels when compared to control cells. Western blotting analysis revealed activation of both caspase-9 and caspase-3 in response to MeHg treatment. Furthermore, astrocytes treated with MeHg showed a significant increase in ROCK-1 cleavage when compared to control cells. There were no significant diferences between control and MeHg-treated astrocytes in the expression of total ROCK-1 protein. Taken together, these findings suggest that the activation of ROCK-1 by caspase-3 in astrocytes may play an essential role in MeHg-induced apoptosis.

1323 Phosphine Gas Inhalation Induces Apoptosis in the Heart in Rats

D. K. Andres[2], B. J. Benton[2], O. C. Clark[2], J. Simons[2], J. A. Tressler[2], J. F. Andres[2], J. Devorak[2], R. Ray[2] and A. M. Sciuto[2]. [1]*Analytical Toxicology Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD and* [2]*Research Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

Phosphine gas (PH3), a hydrolysis product from the common metal phosphide pesticides (e.g., aluminum phosphide) is colorless, flammable, and highly toxic. The main target organ for PH3 is the lung; however, it is also known to affect the heart, the gastrointestinal tract, and the kidneys in addition to many other systemic effects. Currently, there is no effective medical countermeasure for complications resulting from PH3 exposure. The exact mechanisms of PH3 toxicity are still unclear but are thought to involve inhibition of cytochromes in mitochondria. Interestingly, PH3 toxicity mimics many of the traditional chemical warfare agents, such as vesicants (hemorrhagic lung), nerve agents (cholinesterase inhibition), and phosgene (acute respiratory distress syndrome). Cytotoxicity and apoptosis have been shown to be involved in the mechanism of airway injuries (e.g., due to sulfur mustard inhalation). Therefore, we examined apoptotic changes in the lung, heart, and brain stem at 1, 6, and/or 24 hr following PH3 gas exposure (15480 and 16500 ppm x min) in our rat head-only inhalation model. We observed that PH3 inhalation significantly ($p < 0.05$) increased caspase-3, -9, and -8 in the heart (caspase-3 and -9 are more prominent than -8). However, there were no significant changes in any of the caspases in the lung or the brain stem. For the higher dose, an increase in caspase activity was seen in the heart after PH3 exposure as early as 1 hr for caspase-9 and -8 and 6 hr for caspase-3. These results suggest that the intrinsic or mitochondrial apoptotic pathway may be activated. The PH3-induced apoptosis and its time-course agree with the genomic analysis results showing alterations in both metabolic and immune/inflammatory cellular processes in cardiac tissue. PH3-exposed heart tissue was also examined by transmission electron microscopy (TEM); TEM indicated ultrastructural damage to the myocardium and mitochondria. Our findings suggest a strategy to study the mechanism of PH3 poisoning with respect to the acute cardiopulmonary crisis and, therefore, the development of medical countermeasures.

1324 Enhanced Radiation-Induced Cell Killing Through Modulation of Autophagic Flux in Prostate Cancer Cells

T. A. Thompson[3], T. Borunda[3], T. Anderson Daniels[3], D. MacKenzie[3], K. White[3] and J. Norenberg[3]. [1]*Center for Isotopes in Medicine, University of New Mexico, Albuquerque, NM;* [2]*Internal Medicine, University of New Mexico, Albuquerque, NM and* [3]*Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

Autophagy, a survival mechanism for many cancers, is being considered as a viable target for cancer chemotherapy. A drug screen identified astemizole, a second-generation antihistamine, as a potent modulator of autophagy in androgen-sensitive (e.g., 22Rv1 and LNCaP cells) and androgen-independent (e.g., PC3 cells) prostate cancer cell lines. In this study, the action of astemizole to potentiate radiation-induced cell death in prostate cancer cells was investigated to address the hypothesis that combined radiation and astemizole treatment synergistically produce cell death through an autophagic flux mechanism. Immunoblotting showed that astemizole and radiation synergistically increased LC3 II protein expression, a biomarker for autophagy. Modulation of autophagy by astemizole was further confirmed by acridine orange staining and punctae formation in PC3 cells expressing LC3-GFP. Cell death induced by astemizole was time-dependent showing successively lower TD50's of 25, 12, and 8 micromolar at 24, 48, and 72 h, respectively, in PC3 cells. Further, 5 Gy gamma-radiation reduced cell viability to 50% at 24h. MTS analysis showed at least additive effects with combined 8 micromolar astemizole and 5 Gy radiation treatment. Combining the results from flow cytometric and hemacytometric analyses provided evidence for a necrosis-based mechanism of cell death induced by astemizole. Importantly, cell fragmentation was found to be an accurate measure of both astemizole and radiation induced cell death. Using these methods, it was found that the order of radiation treatment followed at 24h by astemizole treatment was optimal to produce enhanced cell death. Our results suggest that modulators of autophagy combined with radiation lead to at least an additive effect for prostate cancer cell killing and a synergistic induction of autophagy.

1325 Cytotoxicity of Whole Smoke Conditioned Media Generated from 3R4F Reference Cigarettes to Human Aortic Endothelial Cells

O. Makwana[2], H. Flockton[1], G. Watters[1], W. Fields[2] and B. Bombick[2]. [1]*Genetic and Molecular Toxicology, Covance Laboratories Ltd, Harrogate, United Kingdom and* [2]*Research and Development, R.J. Reynolds Tobacco Company, Winston-Salem, NC.* Sponsor: R. Potts.

Chronic exposure to cigarette smoke can lead to endothelial dysfunction and ultimately endothelial cell death, which is often associated with atherosclerosis. Here, we are utilizing Human Aortic Endothelial Cells (HAECs) from healthy donors to determine smoke-induced cytotoxicity. Neutral red uptake (NRU) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assays were used to evaluate the cytotoxic response of whole smoke conditioned media (WSCM) in four donors of HAECs. WSCM was generated from four Kentucky Reference 3R4F cigarettes smoked under ISO regime then bubbled into 30 mL of endothelial cell culture growth media using glass impingers. The WSCM media (containing 2% BSA) was then filtered at 0.10 µm. HAECs were exposed to WSCM over a range of concentrations in each experiment. After a 24-hour exposure, NRU and/or reduction of MTT to formazan was determined for each treatment concentration and compared to control cultures. The IC50 (the concentration producing 50% reduction of either neutral red uptake or formazan formation) was calculated from the concentration-response. For a dose range of 0-8000 ng/mL nicotine equivalence (n.e.) of WSCM, the following IC50 values in the NRU assay were observed: 4721 ng/mL n.e. ± 1078 for donor A, 4731 ng/mL n.e. ± 966 for donor B, 5172 ng/mL n.e. ± 1406 for donor C and 4827 ng/mL n.e. ± 320 for donor D. Using the same dose range of 0-8000 ng/mL n.e. of WSCM, the following IC50 values in the MTT assay were observed: 5113 ng/mL n.e. ± 1356, 4724 ng/mL n.e. ± 846, 5769 ng/mL n.e. ± 645 and 5275 ng/mL n.e. ± 654 for the four donors. In summary, the IC50 data confirmed that the cytotoxic response to the test article was similar for HAECs from each donor. We conclude that WSCM produces comparable cytotoxicity *in vitro* when applied to HAECs from four donors as assessed using both the NRU and MTT assays.

1326 Targeting Acute Promyelocytic Leukemia Using Prolonged Low Doses of Cisplatin

S. Dasari, C. Yedjou, V. R. Velma and P. B. Tchounwou. *Biology, Jackson State University, Jackson, MS.* Sponsor: P. Tchounwou.

Acute promyelocytic leukemia (APL) is a blood cancer that accounts for approximately 10% of all acute myeloid leukemia cases. Chromosomal mutations are one of the major causes for this cancer. Approximately 1.2% of cancer deaths in the United States are due to APL. Cis-diamminedichloroplatinum (II) (cisplatin) is the most widely used chemotherapeutic drugs, but its effectiveness is limited by tumor cell resistance and the severe side effects it causes. Chemotherapy with cisplatin causes a wide range of side effects including nausea and vomiting, hair loss, and fatigue. Since high level of cisplatin is cytotoxic to both cancer and normal cells, the goal of the present study was to explore prolonged low doses of cisplatin in the management of leukemia using HL-60 cells as a test model. To achieve our goal, human leukemia (HL-60) cells were treated with different concentrations (1µM, 2 µM and 3 µM) of cisplatin for 24, 48, 72 and 96 hours, respectively. Both oxidative stress and oxidative damage were estimated by lipid peroxidation, glutathione peroxidase, catalase, and comet assays, respectively. Data obtained from the MTS assay demonstrated that cisplatin treatment decreased the number of viable tumor cells by direct cell killing or by simply decreasing the rate of cellular proliferation in a dose-and time dependent fashion. The results of the lipid peroxidation showed a significant increase ($p < 0.05$) of malondialdehyde levels with increasing cisplatin doses. Results obtained from glutathione peroxidase and catalase assays showed a gradual decrease in antioxidant enzyme activity in cisplatin-treated cells as compared to the control. Data generated from the comet assay demonstrated a significant dose-dependent increase in DNA damage, with respect to comet tail-length and tail moment, as a result of cisplatin treatment. Taken together, our research demonstrated that cisplatin inhibits the growth of cancer cells at least in part, via induction of oxidative stress and oxidative damage. The research supported in part by a grant from the National Institutes of Health (Grants No. NIMHD-G12MD007581) and in part by the Mississippi INBRE (Grants no. NIGMS-P20GM103476)

1327 Preclinical Studies of Medicinal Plant for the Treatment of Ovarian Cancer

A. T. Mbemi, C. Yedjou, V. I. Ogungbe and P. B. Tchounwou. *Biology, Jackson State University, Jackson, MS.* Sponsor: P. Tchounwou.

Ovarian cancer is classified as the fifth most common forms of cancers and the first leading cause of death among all gynecological malignancies in women. The mortality rate is lower in African-American women than Causasian women. The major contributor to high death rate of ovarian cancer is attributed to the absence of the specific symptoms and the lack of adequate screening of the tumors before they metastasize beyond the ovaries. The development of new drugs from natural products is considered important. *Vernonia amygdalina* (*VA*) is a tropical plant that has been used by African traditional healers for the treatment of many diseases. Therefore, the goal of this research was to determine the therapeutic mechanisms of *VA* leaf extracts in the management of ovarian cancer. To achieve our goal, cell viability was determined by MTS assay using the spectrophometer. Cell cycle and cell apoptosis was measured by flow cytometry analysis. Data obtained from MTS assessment indicated that *V. amygdalina* significantly reduced the number of live cells in a dose-dependent manner. Flow cytometry data demonstrated that *V. amygdalina* induced cell cycle arrest and triggered apoptosis in treated cells compared to the control group. Our research summary demonstrated that *V. amygdalina* destroys ovarian cancer cells by reducing the percentage of cell viability, inducing cell arrest at the G0/G1 checkpoint, and triggering apoptosis through phosphatidylserine externalization, and caspase-3 activation. This result suggests that *V. amygdalina* treatment may be a good anti-cancer candidate for the treatment of ovarian cancer. Research supported by NIH-RCMI Grant # G1200MD007581 at Jackson State University and part by the Mississippi INBRE (NIGMS-P20GM103476).

1328 ASD-49, A Novel Aspirin-Derived Small Molecule, as a Potential Therapeutic Agent for Colorectal Cancer

D. N. Karelia[2], M. K. Pandey[2], D. Plano[1], S. Amin[2] and A. K. Sharma[2]. [1]*Department of Organic and Pharmaceutical Chemistry, University of Navarra, Pamplona, Spain and* [2]*Pharmacology, Penn State College of Medicine, Hershey, PA.*

Colorectal cancer (CRC) is expected to kill over 400,000 people worldwide this year. While the current standard of care can extend the survival of the patients, CRC remains second in the number of cancer related deaths in the industrialized world. Therefore, there is an urgent need to develop effective therapies that will enhance the survival and improve quality of life of CRC patients. Non-steroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, have been shown to be effective in decreasing cancer incidences, including that of CRC, and have shown promising chemopreventive potential. However, prolonged usage and higher doses are associated with gastrointestinal toxicities. In addition, aspirin is not potent enough to be used as a therapeutic agent. Through extensive structure-activity relationship studies based on NSAIDs structures, we have synthesized and identified a novel aspirin derived selenium hybrid molecule, ASD-49, which is >100 times more potent than aspirin in inhibiting the viability of cancer cells. We tested the cytotoxic potential of this novel molecule against various cancer models including CRC. We found ASD-49 to be effective against a variety of cancer cells with an EC50 in the range of 1 to 2.5 µM. Furthermore, it is selectively toxic to cancer cells as compared to normal human dermal fibroblasts (nHDFs). Importantly, our results suggest that anti-cancer potency of ASD-49 is greater than the first line chemotherapeutic for CRC, 5-FU. ASD-49, arrests CRC cells in G1 and G2/M phase of cell cycle, which is accompanied by reduction in cyclin levels and an increase in cell cycle inhibitory protein, p21. Moreover, ASD-49 induces apoptosis in CRC cells via activation of caspase 3/7 and PARP cleavage. Taken together, ASD-49 has a potential to be developed as a novel therapy for CRC.

1329 Nupr1/Chop Signal Axis Is Involved in Mitochondrion-Related Endothelial Cell Apoptosis Induced by Methamphetamine

W. Xie[1], D. Cai[1], D. Qiao[1], X. Yue[1], Q. Wang[1], D. Li[1], Z. Lin[2] and H. Wang[1]. [1]*Forensic Medicine, Southern Medical University, Guangzhou, China and* [2]*Institute of Computational Comparative Medicine and Department of Anatomy and Physiology, Kansas State University, Manhattan, KS.*

Methamphetamine (METH) abuse has been a serious global public health problem for decades. Previous studies have shown that METH causes detrimental effects on the nervous and cardiovascular systems. METH-induced cardiovascular toxicity is, in part, attributed to its destructive effect on vascular endothelial cells. However, the underlying mechanism of METH-caused endothelium disruption has not been investigated systematically. In this study, we demonstrated that METH exposure caused mitochondrial apoptosis in human umbilical vein endothelial cells(HUVECs) and cardiac microvascular endothelial cells(CMECs), which was partly attributable to up-regulated nuclear protein 1 (Nupr1). We found that METH medicated endothelial cell apoptosis through Nupr1/Chop/PUMA signaling pathway. Specifically, PUMA initiated mitochondrial apoptosis by downregulating antiapoptotic Bcl-2, followed by upregulation of proapoptoticBax, resulting in translocation of cytochrome c, an apoptogenic factor, from the mitochondria to cytoplasm and activation of caspase-dependent pathways. Interestingly, increased Beclin upregulated Chop, which together formed a ternary complex with Bcl-2, thereby decreasing the dissociative Bcl-2. As a result, the ratio of dissociative Bcl-2 to Baxwas significantly decreased, which led to translocation of cytochrome c and initiated more drastic apoptosis. These findings were supported by data showing that METH-induced apoptosis was significantly inhibited by silencing Nupr1 or Chop, or by PUMA and Beclin knockdown. Based on these data, a novel mechanistic model of METH-induced endothelial cell toxicity is proposed. Collectively, these results highlight that the Nupr1/CHOP/PUMA pathway is essential for mitochondrion-related endothelial cellapoptosis induced by METH. Keywords: Methamphetamine; Nupr1/Chop/PUMA/Beclin; Apoptosis; Endoplasmic Reticulum Stress (ERS); Endothelial cells

1330 Lead Nitrate-Induced Cell Cycle Arrest, Genotoxic and Apoptotic Effect in Human Leukemia (HL-60) Cells

H. M. Tchounwou, C. Yedjou and P. B. Tchounwou. *Biology, Jackson State University, Jackson, MS.* Sponsor: P. Tchounwou.

In recent years, the industrial use of lead has been significantly reduced from paints and ceramic products, caulking, and pipe solder. Despite this progress, lead exposure continues to be a significant public health concern. Previous study in our laboratory showed that the treatment of human leukemia (HL-60) cells with lead nitrate significantly increased lipid hydroperoxide levels, a major degradation product of unsaturated phospholipids and glycolipids. The main goal of this research was to determine the *in vitro* mechanisms of lead nitrate [Pb(N03)2] induce DNA damage, apoptosis, and cell cycle arrest in human leukemia (HL-60) cells. To reach our goal, HL-60 cells were treated with different concentrations of Pb(N03)2 for 24 hours. Live cells and necrotic death cells were measured by the propidium idiode (PI) assay using the cellometer vision. Cell apoptosis was measured by the flow cytometry and DNA laddering. Cell cycle analysis was evaluated by the by the flow cytometry. The result of the PI demonstrated a significant ($p<0.05$) increase of necrotic cell death in Pb(N03)2-treated cells, indicative of membrane rupture by Pb(N03)2 compared to the control. Data generated from the comet assay indicated a concentration-dependent increase in DNA damage in the nucleic of HL-60 cells, showing a significant increase ($P < 0.05$) in comet tail-length, tail arm and tail moment, and percentages of DNA cleavage with the range concentrations tested. Data generated from the flow cytometry assessment indicated that Pb(N03)2 exposure significantly ($p < 0.05$) increased the proportion of caspase-3 positive cells (apoptotic cells) compared to the control. The flow cytometry assessment also indicated Pb(N03)2 exposure caused cell cycle arrest at the G0/G1 checkpoint. The result of DNA laddering assay showed presence of DNA smear in the agarose gel with little presence of DNA fragments in the treated cells compared to the control. Taken together, our findings suggest that lead exposure significantly ($P < 0.05$) reduces cellular viability, induces DNA damage and apoptosis, and causes cell cycle at the G0/G1 checkpoint in exposed human leukemia (HL-60) cells. The research supported in part by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476 and in part by a grant from the National Institutes of Health (Grant No. NIMHD-G12MD007581).

1331 Molecular Mechanisms of Cisplatin Cytotoxicity in Acute Promyelocytic Leukemia Cells

P. B. Tchounwou. *Jackson State University, Jackson, MS.*

Cis-diamminedichloroplatinum (II) (cisplatin) is a widely used anti-tumor drug for the treatment of a broad range of human malignancies with successful therapeutic outcomes for head and neck, ovarian, and testicular cancers. It has been found to inhibit cell cycle progression and to induce oxidative stress and apoptosis in acute promyelocytic leukemia (APL) cells. However, its molecular mechanisms of cytotoxic action are poorly understood. We hypothesized that cisplatin induces cytotoxicity through DNA adduct formation, oxidative stress, transcriptional factors (p53 and AP-1), cell cycle regulation, stress signaling and apoptosis in APL cells. We used the APL cell line as a model, and applied a variety of molecular tools to elucidate cytototoxic mode of action of cisplatin. We found that cisplatin inhibited cell proliferation by a cytotoxicity, characterized by DNA-adduct formation, oxidative stress, cell cycle arrest, stress signaling and apoptosis in APL cells. Cisplatin also activated p53 and phosphorylated activator protein (AP-1) component, c-Jun at serine (63, 73) residue simultaneously leading to cell cycle arrest through stimulation of p21 and down regulation of cyclins and cyclin dependent kinases in APL cell lines. It strongly activated the intrinsic pathway of apoptosis through alteration of the mitochondrial membrane potential, release of cytochrome C, and up regulation of caspase 3 activity. It also down regulated the p38MAPK pathway. Overall, this study highlights the molecular mechanisms of cisplatin in APL toxicity to APL cells, and provides new insights on the novel targets to overcome APL disease and/or to design new APL drugs. Key words - Cisplatin, APL cell line, cell cycle modulation, apoptosis, AP-1 and p53.

1332 Understanding Anti-CD137-Induced Liver Toxicity

M. Wang[3], B. R. Baumgart[1], F. Simutis[1], W. Freebern[2], K. D. Chadwick[2], R. T. Bunch[1], C. Ju[3] and K. D. Price[2]. [1]*Bristol-Myers Squibb, Mt Vernon, IN;* [2]*Bristol-Myers Squibb, New Brunswick, NJ and* [3]*University of Colorado Health Sciences Center, Aurora, CO.*

CD137 is expressed on cells of the immune system. Anti-CD137 monoclonal antibodies provide co-stimulatory signals to antigen-specific activated T-cells prompting T-cell expansion, cytokine production, and enhanced cytotoxic activity. BMS-469492, the murine homolog of Urelumab, enhances CD8+ T-cell cytotoxic responses. The objective of this study was to investigate potential mechanisms of BMS-469492-induced hepatotoxicity in mice by identifying cellular sources of TNF-α and IFN-γ and determining pathological involvement of natural killer (NK) cells, natural killer T (NKT) cells, and CD8+ T-cells. A series of experiments were conducted wherein 3 weekly doses of BMS469492 at 5 mpk were administered to female wildtype Balb/c mice, CD1d-/- mice with NKT cell deficiency, or wild-type Balb/c mice depleted of CD8+ T-cells. Microscopically, BMS-469492 induced minimal single-cell hepatocyte necrosis, sinusoidal leukocyte infiltration, and minimal to slight chronic inflammation that correlated with increases in serum alanine aminotransferase (ALT) (4.7-11.4) and aspartate aminotransferase (AST) (2.3-5.2) activities. In the liver only, there was a 2 increase in CD45+ cells after the first dose and an 8 increase following subsequent weekly doses. CD8+ T-cells increased over 50-fold in the liver while there was no difference in liver NK or NKT cell numbers. Additionally, administration of BMS-469492 resulted in significantly increased CD3 (27), CD8 (107), IFN (44), and TNF (47) hepatic mRNA expression. BMS-469492-induced hepatotoxicity was similar between wild-type and CD1d-/- mice. In contrast, depletion of CD8+ cells by anti-CD8a significantly decreased the magnitude of liver injury caused by BMS-469492. In addition, CD8 (0.008), CD3 (3.5), IFN (3), and TNF (3) hepatic mRNA expression levels were significantly lower than in BMS-469492-treated wild-type mice (non-CD8+ T-cell depleted). In conclusion, BMS-469492-mediated hepatotoxicity appears to be related to liver-localized increases in CD8+ T-cells and IFN-γ that can be abrogated by depleting CD8+ T-cells.

1333 Investigation of the Mechanism of Idiosyncratic Clozapine-induced Agranulocytosis Using a Rat Model

A. S. Johnston. *Phamacy, University of Toronto, Toronto, ON, Canada.* Sponsor: J. Uetrecht.

Clozapine (CLZ) is a very effective antipsychotic agent, but its use is limited by the fact that it causes agranulocytosis in 0.1-1% of patients. Although most patients do not develop agranulocytosis, most do develop transient paradoxical neutrophilia and an increase in cytokines such as IL-6. A recent paper also reported an increase in peripheral monocytes that precedes the increase in neutrophils (1). In addition,

we found that treatment of THP-1 cells (a human monocyte cell line) with clozapine led to inflammasome activation with release of IL-1ß. This suggests that most patients do have an immune response to the drug, but in most patients this resolves without resulting in agranulocytosis, presumably via immune tolerance. If we could reproduce this immune response in animals it would allow us to study the mechanism by which clozapine induces an immune response and this could help us understand the mechanism of idiosyncratic agranulocytosis. Mice are very sensitive to the neurologic effects of CLZ. The dose of CLZ that they tolerate is limited, and they do not respond with neutrophilia. In contrast, treatment of rats with CLZ (0.5 mg/ml + 0.1 g sucrose in drinking water) results in clinically relevant serum concentrations of CLZ (0.2 µg/ml), and this did result in neutrophilia. In addition, there was an increase in the % peripheral monocytes (CD3- and CD11b/c+) from 12 to 26%. There also appeared to be an increase in NK cells. Given the result with THP-1 cells, we postulate that these effects are due to activation of monocytes with the production of IL-1ß; however, we were not able to detect an increase in serum IL-1ß in rats. Further studies will be performed to phenotype the monocytes in the rat model and to determine the mechanism by which their numbers are increased. This research was supported by grants from the Canadian Institutes of Health Research. 1. Lee, J., Takeuchi, H., Fervaha, G., Powell, V., Bhaloo, A., Bies, R., & Remington, G. (2015). The Effect of Clozapine on Hematological Indices: A 1-Year Follow-Up Study. Journal of clinical psychopharmacology. 35(5): 510-516.

1334 Alterations of Tnf-Mediated Leukocyte Recruitment in Trovafloxacin-Treated Mice

G. Giustarini, L. Kruijssen, J. Smit, R. Bleumink, M. Bol-Schoenmakers, M. van Roest and R. Pieters. *Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands.*

Idiosyncratic drug-induced liver injuries (DILI) have a poorly understood pathogenesis. Since these injuries are associated with inflammatory stress in human as well as in animal models we investigated the kinetics of innate immune changes during development of DILI. In this study, we used the trovafloxacin (TVX)-induced liver injury protocol as established by Shaw et al (Tox Sci 2009). Briefly, mice were administered by gavage with TVX (150 mg/kg), its pharmacological analogue LVX (375 mg/kg) or the vehicle, and after 3 hours they were injected with TNF (50 µg/kg, ip). Mice were sacrificed before TNF injection (0h), and 2 (2h) or 4 hours (4h) after the cytokine challenge. TVX and TNF co-administration led to massive liver damage, increased serum levels of ALT, and production of cytokines IL6, TNF, MIP2 and IL10. Accumulation of neutrophils at 4h after the TNF injection was significantly higher in TVX-exposed livers when compared to the other treatments. In addition, the number of apoptotic cells in TVX-exposed livers already increased before TNF injection. Remarkably, at 2 h after TNF injection the recruitment of neutrophils and inflammatory monocytes was significantly lower in the liver of TVX-treated mice when compared to those obtained from mice receiving LVX or the vehicle only. Interestingly, this reduced recruitment was accompanied by a reduction of ICAM1 expression on liver tissue in case of TVX, whereas the production of the neutrophil chemoattractant MIP2 was not reduced. Moreover, 2h after TNF administration an increased influx of neutrophils and monocytes was observed in LVX-exposed livers when compared to those in vehicle treatment. From these data we conclude that TVX pre-treatment leads to dysregulation of TNF-induced leukocyte recruitment in the liver. Unresponsiveness to inflammatory stimuli and reduced clearance of damage might be involved in TVX-induced liver injury.

1335 Early Juvenile Exposure of Mice to High-Dose Acetaminophen Confers Elevated Serum Eotaxin Levels and Sociability Impairments in Adulthood

M. T. Edwards[2], G. G. Gould[2], R. Alvarez[1] and S. T. Schultz[2]. [1]*Naval Medical Research Unit, Ft. Sam Houston, San Antonio, TX and* [2]*Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX.*

Based on epidemiological evidence, exposure of children under 5 years old to acetaminophen may be problematic for social brain development if it can't be properly metabolized. We have used high dose acetaminophen exposures in juvenile mice (P18-21) to explore mechanisms that might compromise sociability in this population of susceptible children. The study hypothesis was that high-dose (>100 mg/kg) acetaminophen exposure in weanling (PD18-21) mice might produce impairments in the social behavior of C57BL/6J mice, and would worsen them in socially deficient BTBR mice. Male C57BL/6 and BTBR mice were exposed for 4 days to injections of acetaminophen (400 mg/kg) or vehicle (saline + 10% DMSO), and matured to adulthood (PD 80). Three chamber sociability tests of C57BL/6 mice revealed a significant ($p<0.05$) reduction in social interaction relative to vehicle controls that was comparable to prenatal treatment with valproic acid. However, the social behavior of BTBR mice was unaffected. We postulated that persistent adverse immune responses might have contributed to this outcome. Serum collected after the behavior tests was used for cytokine and chemokine measurements performed using the Bio-plex mouse cytokine 23-plex panel on the Bio-plex 200 suspension array system. Eotaxin levels were significantly higher in C57BL/6 adults treated as juveniles with high dose of acetaminophen. These mice also had high levels of TNF-α, a proinflammatory cytokine, and G-CSF, or granulocyte colony-stimulating factor. In contrast, in BTBR mice, eotaxin levels were low and along with G-CSF, they were unaffected by acetaminophen. TNF-α expression was reduced in acetaminophen treated BTBR relative to controls. Eotaxin can inhibit neurogenesis, particularly in the hippocampus. We therefore looked for evidence of reduced hippocampal serotonergic neuronal density based on saturated serotonin transporter binding by quantitative autoradiography, but we found no difference from control treatment values. However, the elevated eotaxin levels in C57BL/6 treated as juveniles with high-dose acetaminophen and differences among strains in their levels may relate in other ways to their sociability phenotypes.

1336 Direct Effects of Ethanol at Toxicologically Relevant Concentrations on TLR3 Conformation and Ligand Binding

W. Tan[2], V. Le[2], E. Lewis[2], S. Gwaltney[2], B. Manikanthan[2], A. Shack[2], B. Nanduri[2] and S. B. Pruett[2]. [1]*Department of Basic Sciences, Mississippi State University, Mississippi State, MS and* [2]*Department of Chemistry, Mississippi State University, Mississippi State, MS.*

We reported previously that ethanol changes the conformation of an important immunological receptor, toll-like receptor 3 (TLR3). Here we present new data indicating that ethanol-induced conformational changes in human and mouse TLR3 occurred at concentrations relevant to human exposure, as demonstrated by changes in the circular dichroism spectrum of TLR3 ectodomains. Molecular modeling software (AutoDock 4.2) predicted a single ethanol binding site in both human and mouse TLR3 with a dissociation constant that would produce ethanol binding in the concentration range that also caused conformational changes. However, the ethanol binding sites did not overlap the ligand binding sites in either human or mouse TLR3, so it is unlikely that ethanol acts by steric hindrance. Rather, if the ethanol binding site is functionally important, it must act allosterically. Actually, allosteric effects mediated through an ethanol binding site or by altering solvent properties in this aqueous system could either interfere with ligand binding or could decrease ligand-induced TLR3 conformational changes that are normally transmitted to the cytoplasmic domain of the TLR3 molecule leading to signal transduction. In the present study, ethanol at concentrations that changed the conformation of TLR3 also significantly decreased the binding of a TLR3 ligand to immobilized TLR3 in a cell-free system. Ethanol at concentrations from 10-100 mM changed the conformation of human and mouse TLR3, allowed occupation of an ethanol binding site (as indicated by a computational method), inhibited binding of ligand to immobilized TLR3, and (as shown in previous studies) inhibited cellular signaling and innate immune responses. It should be noted that relatively few proteins have been reported for which ethanol at relevant concentrations significantly affects conformation and function. Thus, ethanol-induced conformational changes may represent a relatively selective and fundamental mechanism by which ethanol decreases many immunological functions. This work was supported by grant # R01AA009505 from the National Institute on Alcohol Abuse and Alcoholism and by grant # P20GM103646 from the National Institute for General Medical Sciences.

1337 Identification of the Cleaved Klotho Protein That Protects Against Oxidative Cigarette Smoke Induced Cell Death

M. Njenga, T. Lone and D. J. Blake. *Biology, Fort Lewis College, Durango, CO.*

Chronic obstructive pulmonary disease (COPD) is currently the third leading cause of death in the United States. Cigarette smoke (CS) is the primary cause of COPD, although exposure to air pollution, respiratory infections and genetic factors also contribute to disease progression. Exposure to CS induces oxidative stress in the lung, which results in cellular senescence of the lung, leading to decreased proliferation of epithelial cells and the destruction of alveolar structure resulting in pulmonary emphysema. The anti-aging gene, klotho, codes for a membrane bound protein, which is cleaved from the cell surface by ADAM10 and ADAM17, which are α-secretases, and then released as a soluble hormone. Soluble Klotho has been shown to be a key regulator of oxidative

stress, cellular senescence and inflammation. Our group has shown that increased soluble Klotho expression protects against cigarette smoke induced cell death in human epithelial cells through reducing reactive oxygen species levels. Therefore, enhancing Klotho activity in pulmonary epithelial cells, particularly those exposed to CS, may be a promising strategy to alleviate apoptosis and possibly prevent the development of COPD. Klotho contains two major cleavage sites, one close to the juxtamembrane and another between the KL1 and KL2 domains. Currently it is unknown which Klotho isoform leads to cellular protection against CS. Our preliminary results indicate that the full length Klotho protein is expressed at high levels within the membrane and then is efficiently cleaved and released into the cell culture supernatant as determined by immunofluorescence and Western blotting. The larger cleaved protein (~130 kDa; KL1 and KL2) predominates over the smaller (~70 kDa; KL1 only) protein in cell culture supernatants. Our preliminary studies indicate that the N-terminal domain, which can include both the larger and smaller secreted protein, has anti-apoptotic properties using antibody neutralization assays. Currently it is unclear, however, if the KL2 cleaved product has anti-apoptotic properties as well.

1338 E-Cigarette Liquids Alter Human Peripheral Blood Neutrophil, Alveolar Macrophage, and Natural Killer Cell Function

P. W. Clapp, E. A. Pawlak, P. B. Setzer and I. Jaspers. *Center for Environmental Medicine, Asthma, and Lung Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

New and emerging tobacco products, such as flavored e-cigarettes, are advertised as safer alternatives to traditional cigarettes. Although "vaping" is gaining popularity, little is known regarding the health effects of exposure. Moreover, the increasing use of flavored e-liquids is out-pacing toxicological studies of these products. To assess the effects of e-liquid exposure, we challenged human peripheral blood neutrophils (PMNs), natural killer (NK) cells, and alveolar macrophages, with seven flavored e-liquids. These cells were chosen based on their importance in respiratory mucosal host defense responses. Following exposure, markers of cytotoxicity, inflammation, and cellular function were determined for all cell types. PMN function was evaluated by neutrophil extracellular trap (NET) formation, while the phagocytic capacity of PMNs and alveolar macrophages was assessed using pH-sensitive, fluorogenic *Staphylococcus aureus* bioparticles. A flow cytometry-based killing assay quantified NK cell activity. All tested e-liquids altered cellular responses in the leukocyte populations studied. In particular, "Kola" and "Sini-cide" induced cytotoxicity in PMNs, while all e-liquids, except "Solid Menthol", increased proinflammatory IL-8 secretion. "Sin-a-cide" induced nucleic acid release from PMNs, while other flavors had no effect on, or suppressed NET formation. All flavors, except "Kola" and "Solid Menthol", decreased PMN phagocytosis, while only "Sini-cide" impaired phagocytosis in alveolar macrophages. NK cell cytotoxic function varied by e-liquid; "Kola" and "Sini-cide" had a suppressive effect while "Solid Menthol" and "Menthol Tobacco" increased NK cell killing. These results suggest that e-liquid flavor compositions contribute to the variable cellular responses observed, with flavors such as "Sini-cide" having adverse effects across all cell types and endpoints examined. Based on these results, we plan to further investigate adverse responses to common e-liquid flavoring components and correlate exposure with specific physiological outcomes.

1339 Crack Cocaine Effect on the Immune Responses of Rats Exposed to its Smoke, Twice a Day, for 28 Days

I. M. Hueza[1,2] and F. Ponce[2]. [1]*Federal University of São Paulo - UNIFESP, Diadema, Brazil and* [2]*School of Veterinary Medicine and Animal Science - University of São Paulo, São Paulo, Brazil.* Sponsor: K. Welch.

Background: Crack cocaine has been used indiscriminately worldwide. Although several immunotoxic effects in human and animals with other forms of cocaine, there are no toxic reports of the pulmonary exposure to crack smoke on the immune responses of rats. Thus, the aim of this study was to evaluate the immunotoxic effects of crack in rats exposed twice a day for 28 days. Methods: Twenty male rats were equally divided into a control (Co) and a crack cocaine group (CR). CR group was exposed during 10min twice a day to the smoke resulted from the burning of 250mg of crack (cocaine blood level = 175ng/mL) confined into an acrylic device developed in our lab for 28 consecutive days. Co group was only confined into the device for the same time and period. Food consumption (FC) and body weight gain were recorded every day, since it is known that cocaine promotes anorexic effects. On experimental day 14 and 21, all animals were sensitized with an antigenic protein (KLH) and 7 days later they were challenged with the KLH on the hindpaw. Twenty four hours later, all animals were anesthetized to blood collection for anti-KLG immunoglobulin titers (IgM and IgG) and the evaluation of the delayed type hypersensitivity (DTH) reaction developed on the hindpaw. Results: CR group revealed a reduction on the FC when compared to Co group; however, this effect was transient, revealing that repeated exposure to cocaine can cause a neuronal adaptation and a tolerance process to the anorexic effect. When humoral immune response was assessed, no statistical differences were observed on IgM- and IgG-anti-KLH titer among the groups. Moreover, crack exposure did not compromise the DTH development on rats. Conclusions: Despite several reports of increased infectious diseases among crack users, this study did not reveal any immunomodulator effect of the drug. One possible explanation for this fact may be related to the frequency of exposure that users do which can be up to 25 crack rocks per day. Fapesp: proc n. 2012/24550-7

1340 Δ9-Tetrahydrocannabinol (THC) Inhibits Early Response to Cpg-odn-Mediated Activation of Human Plasmacytoid Dendritic Cells (pDC)

J. E. Henriquez[2], M. Rizzo[2], M. Schultz[2], R. B. Crawford[2] and N. E. Kaminski[2]. [1]*Michigan State University, East Lansing, MI and* [2]*Cell and Molecular Biology, Michigan State University, East Lansing, MI;* [3]*Institute for Integrative Toxicology, Michigan State University, East Lansing, MI;* [4]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI.* Sponsor: N. Kaminski.

Plasmacytoid dendritic cells (pDC) compose a minority of circulating peripheral blood mononuclear cells (PBMC) and bridge the innate and adaptive immune response to viral threat through secretion of interferon α (IFNα) and activation of T cells. Δ9-Tetrahydrocannabinol (THC) is an established immune-cell modulator and believed to mediate disease state exacerbation via suppression of interferons. The objective of this study was to determine whether THC impairs IFNα secretion by pDCs and if so, what intracellular pathway(s) is being affected. Naïve human pDCs were purified (>98%) from PBMCs by negative selection. IFNα secretion was induced by CpG-ODN (15µg/ml) and IFNα secreting pDC's were quantified by flow cytometry. pDCs demonstrated significantly higher expression of Cannabinoid Receptor 2 (CBR2) than CBR1 (P=0.04). pDC's demonstrated significant CpG-ODN-dependent IFNα secretion (P<0.001), compared to controls. pDC secretion of IFNα was suppressed in a concentration-dependent manner by THC (P0.05), a low affinity CBR1/2 ligand. THC significantly suppressed IFNα secretion in isolated CpG-ODN-activated pDCs compared to control (P=0.03). This observation was confirmed by measuring the concentration of secreted IFNα and expression of IFNα mRNA. THC also suppressed CD83, a critical co-stimulatory molecule for T cell activation and pDC maturation marker, (P<0.05). TLR-9 associated signaling events; Interferon Response Factor -7 (IRF-7) phosphorylation and cytosolic retention of Osteopontin (OPN), were equally suppressed by CBD, and THC. This indicates that there is a yet to be discovered mechanism for THC-mediated suppression of early TLR-9-mediated activation of pDCs, which differs from its closely related cannabinoid congener CBD. Collectively, these results further support THC as a potent immunosuppressant with both toxic and therapeutic potential.

1341 Δ9-THC-mediated Suppression of Human Plasmacytoid Dendritic Cell and CD14+ Leukocyte Maturation

M. D. Rizzo, J. E. Henriquez, R. B. Crawford and N. E. Kaminski. *Cell and Molecular Biology, Michigan State University, East Lansing, MI; Institute for Integrative Toxicology, Michigan State University, East Lansing, MI; Pharmacology and Toxicology, Michigan State University, East Lansing, MI.* Sponsor: N. Kaminski.

Δ9-Tetrahydrocannabinol (THC), the major psychoactive component of medical marijuana, has been well characterized as an immunosuppressant in human and murine model systems and is controversial as a therapeutic due to its potential health risks. CD83 and CD86 are key markers of maturation that are expressed by certain leukocytes, including plasmacytoid dendritic cells (pDC) and CD14+ (monocytes/macrophages) leukocytes. Both CD83 and CD86 are critical for induction of T lymphocyte clonal expansion and differentiation. Herein, we investigated the role of THC on CD83 and CD86 levels in human blood pDC and CD14+ leukocytes. In human PBMCs stimulated with CpG-ODN 2216 for 24 hours, the percentage of pDC and CD14+ leukocytes expressing CD83 and CD86 showed a concentration-dependent suppression with THC. To elucidate a potential mechanism of suppression of CD83 and CD86 by THC, the expression levels of the adaptor protein EAT-2 were explored. EAT-2, which is a member of the SLAM family of adaptor proteins, plays a crucial role in cytokine production and expression of co-stimulatory

molecules. After CpG-induced activation for 24 hours, the percentage of isolated pDC expressing P-EAT-2 was decreased in a concentration-dependent manner by THC. Collectively, these findings demonstrate the suppressive effects of THC on the maturation of pDC and CD14+ leukocytes along with a potential mode of suppression (EAT-2). The THC-mediated decrease in pDC and CD14+ leukocyte maturation may result in an impairment of T lymphocyte clonal expansion and differentiation, which is of particular importance for immunocompromised portions of the human population (e.g. cancer and HIV patients).

1342 Increased Dendritic Cell Population and Antigen Presenting Function of CB1-/-CB2-/- Derived Mouse Bone Marrow Cells

J. E. Suarez-Martinez[2], R. B. Crawford[2] and N. E. Kamisnki[2]. [1]*Comparative Medicine and Integrative Biology, Michigan State University, East Lansing, MI and* [2]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Our laboratory previously described the immune homeostatic function of cannabinoid receptors (CBRs) using CB1/CB2 null (CB1-/-CB2-/-) mice. CB1-/-CB2-/- mice challenged with influenza exhibited a stronger basal response, greater influx of immune cells and faster clearing of virus. In subsequent *in vitro* studies, dendritic cells (DC) from CB1-/-CB2-/- mice were incubated with OVA257-264 peptide and co-cultured with a OT-I transgenic mice containing T cells that recognize the specific peptide in the context of the major histocompatability complex I (MHC I). DCs from CB1-/-CB2-/- mice induced a CD8+ T cell response in the absence lipopolysaccharide (LPS)-induced DC maturation. The objective of the present study is to characterize the role of CBRs on the development and function of DC subsets from mouse bone marrow. Bone marrow (BM) was extracted from femurs and tibias of WT (C57Bl/6) and CB1-/-CB2-/- mice, stimulated with LPS (1µg/mL) for 24 hours, and stained for DC markers. Our results demonstrate CB1-/-CB2-/- mice have a higher percent of BMDCs in freshly isolated BM and after 24 hours in culture in comparison to WT. BM cells isolated from CB1-/-CB2-/- mice elicited a CD8+ T cell response in the absence of LPS stimulation. Interestingly, there were no differences in MHC I or antigen-bound MHC I complexes on the surface of DCs that can account for this exacerbated activity. CD83, a DC marker implicated in maturation and stimulation of T cells, had a lower expression in DCs from CB1-/-CB2-/- mice. CD83 expressing B cells were identified in the bone marrow and compose ~37% in WT and ~46% in CB1-/-CB2-/- of the total CD83+ population in the BM. This data suggest CBRs are involved in DC development and maturation in addition to mouse BM cellular composition. (This work was supported by DA007908)

1343 β-glucan Administration Enhances Immune Responses through Activation of Regulatory T Cells and Cytokine Production in Mice Exposed to Aflatoxin B1

S. Albakheet, S. F. Ahmad and S. M. Attia. *Pharmacology and Toxicology, King Saud University, Riyadh, Saudi Arabia.*

Aflatoxin B1 (AFB1) is immunotoxic to animals and is a suspected immunosuppressant in humans. β-1,3-Glucan (BG) consists of glucose polymers and has a variety of stimulatory effects on the immune system. In this study, we investigated the role of BG on the expression of phenotypic markers and cytokine secretion in mice exposed to AFB1. We treated animals with BG (150 mg/kg, p.o., once daily) for 7 days beginning at the onset of AFB1 exposure. Exposure of animals to AFB1 alone (1250 µg/kg, i.p, once daily) for 7 days resulted in a decrease in the percentages of lymphocyte subsets (CD4+, GITR+, CD8+, TCR β+, CD3+, Foxp3+, CD4+Foxp3+, and CD127+) as compared to an untreated control (NC). However, both BG alone and BG given in conjunction with exposure to AFB1 significantly increased the percentages of these lymphocyte subsets in heparinized blood. We also observed that mice exposed to AFB1 showed reduced IL-2, TNF-α, IL-17, and IFN-γ production in the spleen and serum. In contrast, oral administration of BG alone and in conjunction with AFB1 exposure augmented the levels of these cytokines. Moreover, this finding was confirmed through RT-PCR and western blot analysis of mRNA and protein expression in the spleen. Altogether, it can be concluded from these studies that BG enhances the responses of lymphocyte subsets, including cytokine production, even when given following exposure to AFB1 immunotoxin.

1344 The Food Preservative tBHQ Suppresses Expression of Il-2 And CD25 Independent of Nrf2 in Human Jurkat T Cells

J. W. Zagorski, A. E. Turley, T. P. Maser and C. E. Rockwell. *Michigan State University, East Lansing, MI.*

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that is activated by cell stress, such as electrophilic compounds and other xenobiotics. In the presence of cell stress, Nrf2 accumulates in the nucleus, where it drives the transcription of target genes, many of which are cytoprotective in nature. We have recently shown that the synthetic food preservative, tBHQ, inhibits several events of T cell activation. Specifically, we found that tBHQ suppresses induction of the early cytokine IL-2, decreases expression of the surface marker CD25, and inhibits transactivation activity of the transcription factor NFκB. The purpose of the current studies was to determine if the immunomodulatory effects of tBHQ are dependent upon Nrf2. Toward this end, several Nrf2-null clones were generated using CRISPR-CAS9 technology, utilizing two different guide RNAs. After generation of the Nrf2-null clones, it was determined that the suppression of IL-2 and CD25 is largely independent of Nrf2. Further studies will be needed to determine the role of Nrf2 in regulating the early events of human Jurkat T cell activation. (This work is funded by NIH grants ES018885 and GM092715).

1345 Protection of Vitamin C and Vitamin E Against Adverse Effects of Phthalate in RAW264.7 Macrophage Cell Line

J.-F. Chang[2], J.-H. Yang[2], C.-Y. Tseng[2] and M.-W. Chao[2]. [1]*Biomedical Engineering, Chung Yuan Christian University, Taoyuan, Taiwan and* [2]*Bioscience Technology, Chung Yuan Christian University, Taoyuan, Taiwan.* Sponsor: M.-W. Chao.

Di-(2-ethylhexyl) phthalate (DEHP) is the most common phthalate plasticizer in plastic industry. DEHP enter into human body via inhalation, ingestion and direct contaction. It can be metabolized to Mono-(2-ethylhexyl) phthalate (MEHP) and absorbed through the villi and travel in the circulation system. Research has indicated that phthalates might cause chronic airway disease including asthma with stimulating IgE and IgG1 production, histamine release, cytokines IL-6 and IL-8 secretion, and generation of oxidative stress. While reactive oxygen species (ROS) is implicated as the cause of these phthalate-induced inflammatory responses. In order to reduce the MEHP-induced oxidative stress and the sequential abnormal inflammation in the murine macrophage Raw264.7 cell line, we treated water-solute vitamin C (Vit C) or lipid-solute vitamin E (Vit E) simultaneously with MEHP. Results showed that DEHP has extremely low cytotoxicity, but MEHP cause dose and time dependent cell death to the cell. Cm-H2DCFDA method indicated that MEHP causes intracellular ROS generation, which triggers an inflammatory immune response causing macrophage activation. In addition, western blot analysis shows that antioxidative enzymes SOD, catalase and HO-1 were up-regulated as well. Next, morphological change in MEHP-activated macrophages was observed in a higher degree of spreading, an increase in their size and the number of the intracellular vacuoles. Our data also demonstrated that MEHP stimulated-macrophages displayed a remarkable increase in extracellular ROS generation, nitric oxide production, and cytokines (TNF-α, TGF-β, IL-1α, IL-1β, IL-6, IL-10, IL-12, and MCP-1) release as compared to the inactivated cells. It was noted however that simultaneous treatment of Vit C or Vit E shows the protection against MEHP induced intracellular free radical production and the subsequent abnormal immune responses.

1346 Upregulation of IgM Production by the Nrf2 Activator tBHQ in LPS-activated Mouse Splenocytes

J. Bursley[2], T. Dunivin[2], S. Umthong[2], J. Zagorski[2], A. Turley[2], H. Dover[2] and C. Rockwell[2]. [1]*Cellular & Molecular Biology Program, Michigan State University, East Lansing, MI and* [2]*Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that is activated by oxidative and electrophilic stress. Recent studies show that Nrf2 plays a role in B cell differentiation. Tert-butylhydroquinone (tBHQ) is a common food preservative and is also used experimentally as an Nrf2 activator. The purpose of the present studies was to determine the effect of tBHQ on B cell function in LPS-activated murine splenocytes from wild-type and Nrf2-null mice. Isolated splenocytes were treated with tBHQ for 30 min prior to activation with LPS. 48h after activation, total IgM production as well as expression of various activation

and plasma cell markers was quantified. Interestingly, tBHQ caused a modest increase in IgM production at low concentrations (0.1 - 1 μM) by wild-type, but not Nrf2-null, splenocytes. Additionally, LPS-activated splenocytes from Nrf2-null mice produced significantly less IgM than wild-type, suggesting that Nrf2 upregulates IgM production by splenic B cells. In both genotypes, tBHQ markedly decreased CD22/CD138 expression while only modestly decreasing CD69/CD25 upregulation. The induction of CD22, CD69, and CD25 was similar between wild-type and Nrf2-null splenocytes. Collectively, this study suggests a novel role for Nrf2 in the regulation of B cell IgM production. (This work is supported by NIH grant: ES018885.)

PS 1347 Lipid Antigen Presentation for T Cell Activation Is Inhibited by Polycyclic Aromatic Hydrocarbons

S. Huang. *Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH.*

Environmental pollution from fuel or coal burning, tobacco smoking, and chemical contamination in barbecued or smoked foods commonly release polycyclic aromatic hydrocarbons (PAHs) and have been associated with multiple human inflammatory diseases and cancers. It is intriguing to speculate that the lipophilic nature of PAHs likely interferes with the production of lipid metabolites and lipid antigen presentation for T cell activation, thus, interrupts the homeostatic inflammatory regulation. To test this hypothesis, we determined the impact of PAHs on the expression of human lipid antigen presenting molecules, including CD1a, CD1b, CD1c, CD1d, and the T cell responses to the lipid antigen presentation. Indeed, our results strikingly demonstrated a broad inhibitory effect of PAH compounds, including Benzo(α)Pyrene, Pyrene, the metabolite 1-HydroxyPyrene, and PAH-containing diesel exhaust particle extracts, on the CD1 surface expression on human dendritic cells and the activation of lipid-reactive T cells in a dose-dependent manner. Moreover, the PAH-exposed dendritic cells further suppress the secretion of cytokine IFN-γ by lipid-reactive T cells. To determine the involving antigen presentation mechanisms, we generated the expression profiles from human dendritic cells and demonstrated that gene expression related to multiple pathways, including lipid metabolism and endocytic antigen loading pathways, is broadly inhibited by Benzo(α) Pyrene. Thus, our data provide a fundamental mechanism that the lipid antigen presentation is inhibited and the activation of lipid-reactive T cell subsets is suppressed upon the co-existence of PAH pollutants with human dendritic cells to support the immunotoxicity mediated by PAH exposure for further interrupting inflammatory homeostasis and worsening diseases. Acknowledgements: This project is supported by NIEHS grant 5P30ES006096 and NIEHS Center of Environmental Genetics Next generation biomedical investigator award.

PS 1348 Effect of Carbamate Pesticides on Human Natural Killer Activity

Q. Li, M. Kobayashi and T. Kawada. *Department of Hygiene and Public Health, Nippon Medical School, Tokyo, Japan.*

Carbamate pesticides are widely used throughout the world in agriculture as fungicides and insecticides. It has been reported that exposure to carbamate pesticides statistically significantly increased risk of non-Hodgkin's Lymphoma in humans suggesting that carbamate pesticides may cause impairments of human immune system because natural killer (NK) cells provide host defense against tumors. Based on the above background, we investigated whether carbamate pesticides show toxicity on human NK cells and conducted experiments with NK-92CI cells, a human NK cell line. We previously confirmed that NK-92CI cells express CD56, perforin, granzymes A, B, 3/K, and grannulysin and are highly cytotoxic to K562 cells in a chromium release assay. NK-92CI cells were treated with carbamate pesticides such as ziram, thiram, maneb, or carbaryl at various concentrations for 2, 4 or 16 h at 37°C *in vitro*. Thereafter, NK activity was determined in a chromium release assay. To explore the mechanism of carbamate pesticide-induced inhibition of NK activity, intracellular level of perforin was determined by flow cytometry. It was found that all carbamate pesticides significantly inhibited human NK activity in a concentration- and time-dependent manner. However, the strength of the effect differed among the pesticides, and the order was thiram ≒ ziram > maneb > carbaryl. Ziram reduced the intracellular levels of perforin in NK-92CI cells in a concentration-dependent manner suggesting that ziram-induced inhibition of human NK activity is at least partially mediated by decrease in the intracellular level of perforin in NK cells. Acknowledgments:This work was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

PS 1349 Dietary Exposure to Low Levels of Mercury Re-Tunes BCR Signaling Sensitivity Potentially Favoring a More Autoimmune Repertoire

A. Rosenspire[2], R. Gill[2] and M. J. McCabe[1]. [1]*Robson Forensic Inc, Lancaster, PA and* [2]*Immunology and Microbiology, Wayne State University, Detroit, MI.*

Upon entering the periphery immature B-1 and B-2 lineage B cells are both referred to as Transitional (T1) B cells. From there they develop into transitional (T2) B cells and then into mature B cells. Phospho-flow analysis of pyCD79a and pySyk during B Cell Receptor (BCR) signaling showed that tuning of BCR signal sensitivity is developmentally regulated. For both B-2 and B-1 lineage B cells, the sensitivity for T1 B cells < mature B cells < T2 cells, although as a group B-1 cells displayed greater sensitivity than B-2 B cells. These findings are consistent with the notion that BCR signal strength controls a negative selective event against self-antigens at the T1: T2 checkpoint, but controls a positive selective event against self-antigens at the T2: mature checkpoint. We suggest that after positive selection at the T2: mature checkpoint, signal sensitivity is tuned down in mature B cells from that in T2 B cells to suppress autoimmune responses arising from cells which have been positively selected. Higher signal sensitivity in B-1 vs. B-2 B cells may be related to suggestions that B-1 cells naturally respond to lower affinity antigens than do B-2 cells. In most instances increases in BCR sensitivity were correlated with increased expression of the phospho-tyrosine kinase Lyn. That is, although as a group B-1 cells had higher Lyn expression than did B-2 B cells, within both the B-1 and B-2 populations, Lyn was maximally expressed in T2 cells and minimally expressed in T1 cells. When Balb/C mice were exposed to low levels of Hg2+ in their drinking water the sensitivity of BCR signaling was reduced in all B cells of either lineage. Counterintuitively exposure to Hg2+ also resulted in decreased phosphorylation of the Y507 tyrosine residue in Lyn. As Y507 is a negative regulatory site of Lyn, decreases in pY507 is functionally equivalent to higher Lyn expression levels. However regardless of the mechanism by which Hg2+ reduces BCR sensitiviey, during B cell development tuning BCR sensitivity downward at the T1:T2 and T2: mature checkpoints would be expected to result in a more autoimmune-like and less diverse BCR repertoire in the resulting mature B cell compartment. Supported by NIH-ES-024476 and NIH-ES-020957.

PS 1350 Developmental Arsenate Exposures Suppress Murine Splenocyte Mitogenesis, without Altering Spleen and Thymus Lymphocyte Population Subsets or Bone Marrow Lymphoid Progenitors

S. Medina[2], S. McClain[2], H. Xu[2], F. T. Lauer[2], D. MacKenzie[2], A. M. Allan[2] and S. W. Burchiel[2]. [1]*Department of Neurosciences, University of New Mexico, Albuquerque, NM and* [2]*Department of Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

Arsenic is a widespread environmental toxicant and common contaminant in drinking water systems. Arsenic exposure has been linked to a multitude of detrimental health effects, including cancer and immunosuppression. Despite the growing awareness of arsenic induced immunosuppression, the immunotoxic effects of early life arsenic exposures remain unclear and require further investigation. The purpose of this study was to examine the function of splenocytes following activation with B cell (LPS) and T cell (Con-A) mitogens, development of bone marrow (BM) lymphoid and myeloid progenitors, and spleen and thymus lymphocyte population subsets in adolescent C57BL6/J mice that were exposed throughout gestation to 500 ppb arsenate. Developmental exposure to 500 ppb arsenate significantly suppressed splenocyte B cell and T cell mitogenesis in male mice; however, in females only B cell mitogenesis was significantly suppressed. BM derived CFU-B and CFU-GM colony formation was not impacted in males or females by the 500 ppb exposure. In the thymus, there were no alterations in CD3+ surface marker defined naïve T cell (CD4+/CD8+ and CD4-/CD8-), helper T cell (CD4+) or cytotoxic T cell (CD8+) population subsets for males or females. T cell subsets were not changed in the spleen and there were also no differences in B cells (CD19+) or monocytes (CD11+/CD49+) for males or females. Collectively, results from this study demonstrate that developmental arsenate exposures can suppress splenocyte B cell and T cell responses to mitogens independent of alterations in lymphocyte population subsets or changes in BM lymphoid and myeloid progenitors. Future studies will focus on exploring comparative effects of developmental arsenite exposures and potential mechanisms of immunotoxicity. This work was supported by RO1-ES019968 (S.W.B.) and RO1-ES019583 (A.M.A.).

1351 A Pilot Study of Arsenic-Induced Immunomodulation of the Prostate Tumor Microenvironment

J. Shearer[2], C. Umbaugh[2], V. Carpio[2] and M. Figueiredo[1]. [1]*Purdue University, West Lafayette, IN and* [2]*University of Texas Medical Branch, Galveston, TX.*

Inorganic Arsenic (iAs) is a well know human carcinogen that has been linked to a variety of cancers, including prostate cancer. While much work has focused on how exposure to iAs can influence tumor progression directly, little has been done to elucidate its effects on the tumor microenvironment (TME). We hypothesize that exposure to iAs creates an environment more conducive for tumor progression by altering cell-cell signaling and immune response in the TME. In order to monitor how exposure to iAs affects stromal-epithelial signaling *in vivo*, we utilized the mouse-derived prostate cancer cell line TC2Ras commixed with adipose-derived mesenchymal stem cells (ASC), which have been shown to be a TME cell population enriched in prostate tumors in patients. Prostate tumors ± ASC were subcutaneously administered to C57/BL6 male mice and supplied with drinking water ± iAs. Upon completion of the study, biological samples were analyzed by flow cytometry, RT-qPCR, and ELISA. Flow cytometry analyses revealed an increase in CD4+ and CD8+ tumor infiltrating lymphocyte populations in the TME in the presence of ASC. RT-qPCR array analyses corroborated flow data by showing substantial increases ranging from 28-119 fold in T-Cell associated gene expression within the TME including Cd28, Cd3e, Cd8a, and IFNγ in the presence of ASC. An intriguing effect of iAs was a global reduction in expression of genes assayed between 14-36 fold compared to control animals which just received regular water. Measurement of IFNγ in serum by ELISA showed a decrease in IFNγ levels in the presence of ASC, suggesting that signaling alterations may be localized to the TME rather than systemically. Our results suggest that exposure to iAs can alter the immune response in the TME by modulating aberrant cell-cell signaling. This is important because it demonstrates that exposure to iAs may increase susceptibility to tumor progression through immunomodulation of the TME.

1352 Environmentally Relevant Concentrations of As+3 and MMA+3 Inhibit IL-7 Signaling in Immature Mouse T Cells

H. Xu, F. T. Lauer, K. J. Liu, L. G. Hudson and S. W. Burchiel. *Department of Pharmaceutical Sciences, University of New Mexico College of Pharmacy, Albuquerque, NM.*

Arsenic exposure from food and drinking water is a worldwide public health issue. Arsenic appears to act via both genotoxic and non-genotoxic pathways to exert its toxicity. Our earlier work demonstrated that pre-lymphoid cells in the bone marrow and thymus are extremely sensitive to *in vivo* and *in vitro* inhibition by low doses of arsenite (As+3) and its metabolite monomethylarsonous acid (MMA+3). In pre-B cells we have evidences showing that IL-7 signaling pathways are impaired by As+3/MMA+3. Therefore, we want to determine if early T cells in the thymus are also affected by arsenic. Immature T cells develop in the thymus, express CD3 first and are double negative (DN) for CD4 and CD8 surface markers. IL-7 is secreted by thymic stromal cells and required for maturation and proliferation of these DN cells. We examined IL-7 signaling in primary DN mouse thymus cells defined by CD makers and a DN mouse T cell line, D1. Inhibition of STAT5 activation (phosphorylation) in primary mouse thymus cells following 18 h *in vitro* exposure to 500 nM As+3 and 50 nM MMA+3 was observed using intracellular antibody staining measured on a flow cytometer. Inhibition of STAT5, JAK1 and JAK3 phosphorylation were also observed in D1 cells exposed to 50 nM MMA+3 and 500 nM As+3 measured by Western Blot. Cell surface expression of the IL-7 receptor (CD127) was also suppressed by 50 nM MMA+3. In summary, the present study clearly indicates that As+3 and its metabolites inhibit IL-7 signaling in pre-T cells at environmentally relevant concentrations *in vitro* and suggests that arsenic may inhibit T cell development in mature mice and, potentially, in developmentally exposed animals. Current studies are aimed at examining downstream effects of the transcriptional factor STAT5 inhibition in IL-7 signaling pathway in DN T cells *in vitro* and the suppression of T cell development following *in vivo* exposures to arsenic in mature mice or during immune system development in pregnant dams. Supported by NIH Grant R01ES019968

1353 Immunotoxic Effects of Sodium Tungstate Dihydrate on Female B6C3F1/N Mice When Administered in Drinking Water

R. Frawley[2], M. Smith[3], K. White[3], S. Elmore[2], R. Herbert[2], R. Moore[1], L. Staska[4], M. Behl[2], M. Hooth[2], G. Kissling[2] and D. Germolec[2]. [1]*EPL, Research Triangle Park, NC;* [2]*NIEHS, Research Triangle Park, NC;* [3]*Virginia Commonwealth University, Richmond, VA and* [4]*WIL Research, Hillsborough, NC.*

Tungsten is a naturally occurring, high tensile strength element that has been used in ammunition, metal cutting, medical devices, and a number of consumer products. Tungsten has been detected in soil, waterways, groundwater, and human tissue and body fluids. Elevated levels of tungsten in urine were reported for populations exposed to tungstate in drinking water in areas where natural formations of tungsten minerals were prevalent. Published reports indicated that sodium tungstate may modulate hematopoiesis, immune cell populations, and immune responses in rodent models. The objective of this study was to assess the potential immunotoxicity of sodium tungstate dihydrate (STD), a drinking water contaminant. Female B6C3F1/N mice received 0-2000 mg/L STD in the drinking water for 28 days, and were evaluated for general toxicity, effects on immune cell populations in spleen and bone marrow, and humoral-mediated, cell-mediated, and innate immunity. Three different parameters of cell-mediated immunity were similarly affected at 1000 mg/L. T-cell proliferative responses against allogeneic leukocytes and anti-CD3 were decreased 32%, and 21%, respectively. Cytotoxic T-lymphocyte activity was decreased at all effector:target cell ratios examined. At 2000 mg/L, the absolute numbers of B220+ (53%), CD11b+ (36%), TER-119+ (50%), and intermediate (22%), high (35%) and total (31%) Gr-1+ progenitor cells in bone marrow were increased; there were no effects on bone marrow DNA synthesis or colony forming capabilities. STD-induced effects on humoral-mediated immunity, innate immunity, and splenocyte subpopulations were limited. Enhanced histopathology did not detect treatment-related lesions in any of the immune tissues. Collectively, these data suggest that exposure to STD in drinking water may adversely effect cell-mediated immunity and hematopoiesis.

1354 The Role of Transcription Factor FoxP3 in the Asbestos-Induced Apoptosis in MT-2 Cells

H. Matsuzaki[1], S. Lee[1], M. Maeda[2], N. Kumagai-takei[1], K. Yoshitome[1], Y. Nishimura[1] and T. Otsuki[1]. [1]*Department of Hygiene, Kawasaki Medical School, Kurashiki, Japan and* [2]*Division of Bioscience, Department of Biofunctional Chemistry, Okayama University Graduate School of Natural Science and Technology, Okayama, Japan.*

It is known that asbestos fibers cause mesothelioma and lung cancer. We previously reported that asbestos affects not only mesothelial and lung epithelial cells, but also anti-tumor immune system. Namely, regulatory T cells, Treg produce inhibitory cytokines to suppress immune reaction against tumor cells. Human T cell line MT-2 cell were cultured with asbestos for 8 months and employed as Treg model exposed to asbestos for long term. MT-2 cells exposed to asbestos showed higher viability after treatment with high concentration of asbestos than original MT-2 cells, and was designated as MT-2Rst. In this study, we found that forkhead transcription factor FoxP3 is down regulated in MT-2 cells after long-term exposure to asbestos. FoxP3 is considered as a master regulator of Treg development. In addition to that, recent study revealed FoxP3 is able to induce apoptosis in Treg. We also found that over expression of FoxP3 induced apoptosis in MT-2 cells. These results indicate that FoxP3 plays important role in regulation of apoptosis induced by asbestos in MT-2 cells. It is plausible that asbestos reduces FoxP3 in normal Treg cells and enhances viability to suppress tumor immune system.

1355 Study for Immunological Importance of Increased Plasma Decoy Receptor 3 Level in Silicosis

S. Lee[4], Y. Nishimura[4], S. Yamamoto[4], T. Hatayama[4], H. Matsuzaki[4], N. Kumagai-Takei[4], K. Yoshitome[4], H. Hayashi[4], K. Uragami[1], M. Kusaka[2], W. Fujimoto[4] and T. Otsuki[4]. *[1]Hinase-Uragami Iin, Okayama, Japan; [2]Kusaka Hospital, Okayama, Japan; [3]Dermatology, Kawasaki Medical School, Kurashiki, Japan and [4]Hygiene, Kawasaki Medical School, Kurashiki, Japan.*

Silicosis patients frequently complicate with autoimmune diseases. Dysregulation of apoptosis, particularly in the Fas/Fas ligand (FasL) pathway, has been considered to play a role in the pathogenesis of autoimmune diseases. DcR3 is known as an intrinsic decoy receptor that binds FasL and inhibits FasL-induced apoptosis. Initial studies showed that DcR3 expression is elevated in cancer cells, however, later work showed that DcR3 expression is also increased in inflammatory diseases, where serum DcR3 levels correlate with disease progression (Linn WW & Hsieh SL 2011). Our previous report showed DcR3 mRNA expression increased in PBMC of silicosis patients (Otsuki T et. al., 2000). At previous this meeting, we reported that plasma DcR3 level in silicosis patients was significantly higher than healthy volunteers'. This result suggested that blood DcR3 increment may cause complication of autoimmune diseases through impair Fas/FasL pathway in silicosis patients. Here we show the results of statistical analysis that addressed a question whether there is correlation between plasma DcR3 level and clinical parameter of autoimmune diseases or plasma cytokines or respiratory score in silicosis patients. Plasma samples that included 19 of healthy volunteers (age 44.84±8.58, men 8: female 11), 20 of silicosis patients (age 74.85±5.35, men 19: female 1), 25 of systemic sclerosis patients (age 62.32±12.06, men 3 : female 22) were provided from Kawasaki Medical School hospital and Kusaka hospital (Bizen, Okayama). All factors were analyzed with ELISA. Statistic analysis was performed using SPSS. There are no correlation between plasma DcR3 and any other clinical parameter of autoimmune diseases in silicosis patients. This result indicated plasma DcR3 elevation means impairment of immune system rather than directing particular autoimmune disease. Furthermore, there are no correlation between plasma DcR3 and respiratory score in silicosis patients. Interestingly, we found a correlation between plasma DcR3 and TGF-b. Further study will be required for clarifying DcR3 importance on immunological alteration in silicosis.

1356 Chronic Exposure to Chrysotile Asbestos Induces Il-17 Production in Cultured Human Peripheral Cd4+ T Cells *In Vitro*

T. Ostuki[2], M. Maeda[1], N. Kumagai-Takei[2], H. Matsuzaki[2], S. lee[2], K. Yoshitome[2] and Y. Nishimura[2]. *[1]Biofunctional Chemistry, Division of Bioscience, Okayama University Graduate School of Natural Science and Technology, Okayama, Japan and [2]Hygiene, Kawasaki Medical School, Kurashiki, Japan.*

To investigate the immunological effects of asbestos, we had established cell line model using an HTLV-1 immortalized human T cell line, MT-2. The continuously exposed subline with low-dose showed the resistance of asbestos-induced apoptosis. The independently established six sublines showed the reduction of surface CXCR3 (sCXCR3) expression and decreased IFNγ production. These findings indicated that asbestos exposure reduce the anti-tumor immunity. In this study, peripheral blood T cells (CD4+, CD4+CXCR3- and CD4+CXCR3+ fractions) were stimulated *in vitro* by anti-CD3/28 and IL-2 with or without chrysotile asbestos co-culture. Thereafter, the intracellular expression of CXCR3, IFNg and IL-17 were analyzed. In addition, IFNg and IL-17 production we well as gene expression of IFNg, IL-17, T-bet and RORgT were analyzed after four hours simulation with Phorbol 12-Myristate 13 Acetate (PMA) and ionomycin (IM). As the results, intracellular IFNg expression was reduced by chrysotile exposure in CD4+ cells, whereas inctacellular IL-17 was increased. The production of IFNg and IL-17 were higher in CD4+CXCR3+ fraction after PMA/IM stimulation, rather than CD4+CXCR3- fraction. In addition, IL-17 production was increased the T cells co-cultured with chrysotile before PMA/IM stimulation. In addition, IL-17 and RORgT gene expressions were increased in all CD4+, CD4+CXCR3- and CD4+CXCR3+ fractions cultured with chrysotile during *in vitro* activation before PMA/IM stimulation. The IL-17 as well as RORgT expressions were higher in CXCR3+ fraction rather than CXCR3- fraction, The enhancement of IL-17 with RORgT may affect tumor-promotion in asbestos exposed patients as the part of presentation for reduction of anti-tumor immunity in these patients.

1357 Alteration of Oxidative Phospholylation-related Complexes in Human T Cell Lines, MT-2 and its Sublines Continuously Exposed to Asbestos Fibers, by Transient Exposure to Asbestos

S. Yamamoto, S. Lee, H. Matsuzaki, N. Kumagai-Takei, T. Hatayama, K. Yoshitome, Y. Nishimura and T. Otsuki. *Hygiene, Kawasaki Medical School, Kurashiki, Japan.*

We have been investigating the immunological effects of asbestos fibers. To address this study focus, we applied an HTLV-1 immortalized human polyclonal T cell line, MT-2 and transient exposure to chrysotile or crocidolite asbestos fibers to MT-2 caused apoptosis with activation of mitochondrial pathway and production of reactive oxygen spaces (ROS) (greater by crocidolite than chrysotile, since their iron-contents). In addition, continuously asbestos exposed sublines of MT-2 were established (designated CB-1 to chrysotile and CR-1 to crocidolite) with obtaining the resistance to asbestos-induced apoptosis. To examine the status of oxidative phosphorylation in original MT-2 (never meet with asbestos), and sublines (CB-1 and CR-1), three days after transient relatively high-dose of asbestos exposure to MT-2 and sublines (sublines were released from continuously exposed fibers for one week), the oxidative phosphorylation related complexes (complex I to V and nicotinamide nucleotide transhydrogenase (NNT) were measured using MILLIPLEX MAP Human Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel - Cellular Metabolism Multiplex Assay. As the results, (1) transient exposure to crocidolite on MT-2 original cells, complex I was reduced, (2) CR-1 revealed higher amounts of complexes II, III, IV and V when compared with original MT-2 cells, whereas CB-2 did not show these tendency, (3), NNT in CB-1 and CR-1 were higher than that of MT-2 original, and (4) complex V was increased after crocidolite exposure in CR-1. These results indicate that, as we reported asbestos fibers induced production of ROS and activation of mitochondrial apoptotic pathway, complex I was reduced among oxidative phosphorylation related complexes, and continuous exposure to crocidolite altered status of these complexes. Future analyses for altered genes expression regarding ROS exclusion.

1358 Effect Of IL-2 Addition on Granzyme B Expression and Proliferation in CD8+ Lymphocytes Exposed to Asbestos During MLR

N. Kumagai-Takei[2], Y. Nishimura[2], H. Matsuzaki[2], S. Lee[2], K. Yoshitome[2], H. Hayashi[2] and T. Otsuki[2]. *[1]Department of Dermatology, Kawasaki Medical School, Kurashiki, Japan and [2]Department of Hygiene, Kawasaki Medical School, Kurashiki, Japan.*

Background and Purpose: Asbestos fibers have tumorigenicity, which is thought to cause mesothelioma and lung cancer. However, its effect on anti-tumor immunity remains unclear. Our previous study reported that chrysotile B asbestos exposure suppressed the induction of CTL during mixed lymphocyte reactions (MLR), accompanied by the decrease in proliferation of CD8+T cells. Therefore, we investigated whether IL-2 insufficiency might contribute to the suppressed induction of CTL upon exposure to asbestos. Materials and Methods: Human PBMCs were cultured with irradiated allogenic PBMCs upon exposure to chrysotile B asbestos at 5 μg/ml for 7 days. After 2 days of culture, IL-2 was added at 1 ng/ml. Flow cytometry was used to analyze expression of cell surface markers and intracellular granzyme, cytotoxicity and proliferation of CD8+ lymphocytes. Results: IL-2 didn't recover the asbestos-caused decreases in numbers of CD3+CD8+ cells, % CD25+ cells, %CD45RO+ cells and increase in %CD45RA+ cells, but partially recovered the decrease in %granzyme B+ cells in CD8+ lymphocytes. The CD8+ lymphocytes from IL-2-added culture with asbestos showed the same degree of cytotoxicity as those from culture without IL-2 and asbestos. IL-2 didn't recover the proliferation of CD8+ lymphocytes during MLR upon exposure to asbestos. IL-2 showed a tendency to increase %granzyme B+ cells in the CFSE-positive CD8+ lymphocytes without proliferation upon exposure to asbestos. Discussion: Those findings indicate that IL-2 insufficiency is not the main cause for the suppressed CTL induction upon exposure to asbestos. The CD8+ lymphocytes from IL-2-added culture showed recovered cytotoxicity, which may be attributed to increase in non-proliferating granzyme B+ cells, not meaning recovered CTL induction. Further studies are needed to clarify the mechanisms of asbestos-caused suppression of CTL induction.

1359 Developmental Activation of the Aryl Hydrocarbon Receptor (AHR) Differentially Modifies Gene Expression Patterns in Naïve and Activated CD8+ T Cells

C. M. Post[1], B. Winans[1], J. Myers[1], W. L. Kraus[2] and B. P. Lawrence[1]. [1]*University of Rochester School of Medicine & Dentistry, Rochester, NY and* [2]*University of Texas Southwestern Medical Center, Dallas, TX.*

Environmental perturbations to the vulnerable developing immune system can lead to dysfunction that persists throughout life; however, the mechanisms by which this occurs remain poorly defined. One mechanism is via the aryl hydrocarbon receptor (AHR), which binds many exogenous chemicals and plays a role in the development and function of the immune system. Developmental exposure to the prototype AHR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes an impaired immune response to influenza A virus (IAV) infection that persists into maturity. CD8+ T cytotoxic lymphocytes (CTL) play a crucial role in fighting acute primary IAV infection due to their ability to directly kill infected cells. Prior to IAV infection, there is no difference in CD8+ T cell frequency between offspring of control and TCDD-treated dams. However, after infection, developmentally exposed offspring have significantly fewer virus-specific CTL than infected offspring of control dams, suggesting that developmental AHR activation interferes with the ability of CD8+ T cells to become CTL. The transition from naïve CD8+ T cell to CTL is a tightly regulated process that involves multiple pathways, including changes in genes that regulate differentiation and proliferation, which could be altered by developmental AHR activation. To determine which genes and pathways are altered, we performed RNA-sequencing on naïve and activated CD8+ T cells from the lymph nodes from offspring of TCDD and vehicle exposed dams. Developmental exposure caused discernible unique changes in the gene expression profiles of naïve and activated CD8+ T cells. Differentially expressed pathways included cell cycle regulation and immune modulation; two key components in the transition from naïve CD8+ T cell to effector CTL. These results provide novel insight into the mechanism by which developmental AHR activation perturbs CD8+ T cell function later in life.

1360 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-Mediated Alterations of EBF1 in Impaired Early Human B Cell Development

J. Li[2,3], A. S. Phadnis-Moghe[3], R. B. Crawford[1,3] and N. E. Kaminski[1,2,3]. [1]*Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI;* [2]*Genetics Program, Michigan State University, East Lansing, MI and* [3]*Institute for Integrative Toxicology, Michigan State University, East Lansing, MI.*

TCDD exposure has been epidemiologically associated with decreased B cell competence and increased incidence of B cell lymphomas. Previous studies have shown that TCDD suppresses antibody responses of human mature B cells; however, the effects of TCDD on early stages of human B cell development from hematopoietic stem cells (HSCs) has not been extensively investigated. To fill this knowledge gap, an *in vitro* human B cell developmental model system was established using human cord blood HSCs. To investigate the effects of TCDD on human B cell development, HSCs were treated with TCDD (1, 10 or 30nM) or vehicle (0.02% DMSO). TCDD-treatment suppressed the expression of the stem cell marker CD34 in a concentration-dependent manner. Moreover, TCDD-treatment decreased the intracellular expression of CD79α, an essential signaling accessory molecule of the B cell receptor, and CD179α, a component of pre-B cell receptor surrogate light chain. Hence, TCDD decreased the generation of CD79α+CD179α+ pro-B cells from HSCs. Given the expression of CD79α and CD179α are regulated by transcription factor Early B-Cell Factor 1 (EBF1), examination of both the mRNA and protein levels of EBF1 showed significant suppression by TCDD. Structure-activity relationship studies demonstrated a correlation between the aryl hydrocarbon receptor (AHR) binding affinity of dioxin-like compounds and the magnitude of CD79α and EBF1 suppression, suggesting the involvement of AHR in TCDD-mediated effects. In addition, binding of the ligand activated AHR to the putative dioxin response elements (DREs) in EBF1 promoter were demonstrated by EMSA-Western assays. Taken together, this study demonstrates for the first time, the impairment of early human B cell development by TCDD, and suggests that transcriptional alterations of EBF1 by AHR are involved in the underlying mechanism.

1361 Aryl Hydrocarbon Receptor Activation in CD11c+ Cells Mediates Effects of TCDD on T Lymphocyte Development in the Thymus

J. M. Kreitinger[2], B. P. Seaver[2], C. A. Beamer[2] and D. M. Shepherd[2]. [1]*Biological Sciences, University of Montana, Missoula, MT and* [2]*Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT.*

Exposure to the environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes immunosuppression. While it has long been known that TCDD exposure results in thymic atrophy, and consequently a reduced T cell repertoire, the mechanisms of action underlying these potent effects are not fully understood. However, activation of the Aryl hydrocarbon Receptor (AhR) in specific immune cell populations appears to be a key mediator. Specifically, we hypothesize that TCDD exposure alters T lymphocyte differentiation indirectly via CD11c+ cells in the thymus. To test this hypothesis, male and female CD11c AhR conditional knockout (AhRCD11c) mice and control (AhRd) mice were gavaged with vehicle or TCDD [100 µg/kg] on day 0. Mice were then injected on day +1 with whole OVA/adjuvant intraperitoneally to induce a systemic antigen-specific immune response, or left unimmunized to assess effects in naïve animals. On day +8 following TCDD exposure, characteristic thymic atrophy (i.e. reduced weight and cell numbers) was observed in AhRd mice exposed to TCDD; however, this effect was significantly ameliorated in AhRCD11c mice. When T lymphocyte developmental stages were assessed, TCDD significantly reduced the frequency of CD4+CD8+ double-positive T lymphocytes in AhRd mice, an effect that was rescued in AhRCD11c mice. Both CD4+ and CD8+ single-positive T lymphocyte populations were increased in AhRd TCDD but not AhRCD11c mice. These effects were observed in both naïve, and immunized mice. Interestingly, double-negative (DN) T lymphocyte development was adversely affected by TCDD, as AhRd mice exposed to TCDD showed an increased frequency of DN stage 1 and 3 thymocytes, while DN stage 4 was significantly reduced. These effects were not observed in TCDD-treated AhRCD11c mice. Collectively, these results demonstrate a significant role for AhR activation in CD11c+ cells as a key event in TCDD-induced disruption of T lymphocyte development in the thymus. This work was supported by grant R01ES013784 from NIGMS/NIEHS.

1362 Aryl Hydrocarbon Receptor Activation Alters Mouse and Human Dendritic Cell Gene Expression Profiles During Influenza A Virus Infection

A. M. Franchini, G. B. Jin and P. Lawrence. *Environmental Medicine, University of Rochester, Rochester, NY.* Sponsor: P. Lawrence.

Ligands of the aryl hydrocarbon receptor (AHR) modulate host responses to pathogens; yet, many details regarding how AHR alters immune cell functions during infection remain undefined. Using influenza A virus (IAV) infection, we previously showed that AHR activation negatively regulates the expansion and differentiation of CD8+ cytotoxic T lymphocytes (CTL), and reduces the ability of dendritic cells (DC) to prime naïve CD8+ T cells. However, the mechanism by which AHR diminishes DC function *in vivo* is unknown. Given that AHR is a ligand inducible transcription factor, unbiased gene expression profiling was used to identify gene and signaling pathways in DCs that are modulated by *in vivo* AHR activation. DCs from infected mice exposed to the prototypical AHR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or control were sorted and used for transcriptome analysis by RNA-seq. This approach affirmed changes in known gene targets, such as indoleamine-2,3-dioxygenase (ido1), which was induced 4-fold, and revealed novel AHR-mediated changes in other genes involved in DC function. Novel affected genes include programmed death ligand 1 (pdl1), which was induced 2-fold; whereas DC-SIGN homolog cd209a and chemokine ccl17 were decreased 3- and 5-fold. We are further extending this work using an *in vitro* human system to compare AHR-mediated gene expression in mouse and human DCs. Human monocyte-derived DCs (hmoDCs) from healthy human donors were exposed to TCDD or control followed by IAV. Targeted transcriptional analyses reveal similar changes in gene expression, including elevated ido1 and decreased cd209a transcript levels. Collectively, this provides evidence that AHR activation in DCs alters gene expression to both enhance immunoregulatory functions and dampen the ability of DCs to properly active naïve T cells. Since these molecules and pathways influence DC function in disease states beyond IAV infection, these findings have broad implications regarding how the AHR regulates DC function.

1363 Differential Effects of the Aryl Hydrocarbon Receptor on Immunoglobulin Gene Expression in Human B Cells

N. L. K. Burra and C. E. W. Sulentic. *Pharmacology and Toxicology, Wright State University, Dayton, OH.*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent and persistent environmental toxin known to inhibit immunoglobulin (Ig) gene expression in various animal models. TCDD is thought to modulate gene expression through the aryl hydrocarbon receptor (AhR) and thereby a subsequent alteration in gene expression. The AhR is a ligand-activated transcription factor that regulates xenobiotic-metabolizing enzymes. The mouse 3'Ig heavy chain regulatory region (3'IghRR) is a sensitive transcriptional target of TCDD that may mediate, in an AhR-dependent manner, the inhibitory effect of TCDD on Ig expression. Human B cells could also be a sensitive target of TCDD. The objective of the current study was to determine the effects of TCDD and the AhR on human Ig expression utilizing a human Burkitt lymphoma cell line (CL-01) model that can be activated to secrete Ig and undergo class switch recombination (CSR) from IgM to IgA, IgG or IgE antibody isotypes. Our results suggest that TCDD has a variable effect on IgM secretion, but significantly inhibits IgG secretion, an effect reversed by addition of the AhR antagonist (CH-223191). Surprisingly, the AhR antagonist alone markedly increased IgG secretion above stimulation. At transcript level, TCDD has variable effects on μ IGH functional transcripts but significantly inhibits g1 (but not g2-4) germline/functional transcripts. Additionally, CD40L and IL-4 stimulation induced *de novo* synthesis of Cε germline transcripts, a precursor to CSR, which was significantly inhibited by TCDD. Interestingly, α1-2 germline/functional transcripts increased in response to TCDD. Notably, TCDD and stimulation had no effect on CYP1A1 expression, which appears to correspond to our recent discovery of SNPs in the transactivation domain (Exon-10) of the AhR. Sequencing suggests that the AhR is heterozygous with one non-functional transactivation domain. Differing effects of the AhR antagonist and TCDD on IGH isotypes suggests the involvement of different mechanisms in the regulation of IGH expression. As the human 3'IGHRR is known to be involved in several autoimmune disorders and also in the regulation of Ig expression, current study could not only help elucidate the role of the AhR in Ig gene expression but also in the relevance of AhR ligands and the 3'IGHRR in autoimmune disorders with a significant antibody component.

1364 Role of Aryl Hydrocarbon Receptor (AHR) Polymorphisms in TCDD-mediated Biological Activity in the Human B Cell

N. Kovalova[2], R. Crawford[2] and N. Kaminski[2]. [1]*Michigan State University, Lansing, MI and* [2]*Pharmacology and Toxicology, Institute for Integrative Toxicology, Michigan State University, Lansing, MI.* Sponsor: N. Kaminski.

2,3,7,8-tetraclorodibenzo-p-dioxin (TCDD) is a high affinity aryl hydrocarbon receptor (AhR) agonist known to suppress antibody responses. Intraspecies variation in sensitivity to the toxic effects of TCDD in rodents is influenced by single nucleotide polymorphisms (SNPs) within the AhR gene. In humans, AhR SNPs might contribute to variation in sensitivity to TCDD-induced suppression of IgM responses. In this study, the functional properties of six (P517S, R554K, V570I, V570I+P517S, R554K+V570I and P517S+R554K+V570I) human AhR variants were examined in the human AhR null B cell line, SKW 6.4. TCDD-induced Cyp1B1 and Cyp1A2 mRNA expression levels and Cyp1B1-regulated reporter gene activity were markedly lower in SKW cells transduced with the R554K AhR variant than in SKW-AHR+ (wild type AhR) cells. DRE-driven reporter activity was not altered by any of the investigated AhR SNPs, suggesting that R554K SNP decreased some but not all transactivation potency of AhR. Furthermore, all AhR variants were able to mediate TCDD-induced suppression of the IgM response; however, a combined P517S+R554K+V570I variant demonstrated a significantly reduced sensitivity to TCDD-mediated suppression of IgM secretion. Collectively, our findings show that the R554K human AhR SNP alone altered sensitivity of human B cells to TCDD-mediated induction of Cyp1B1 and Cyp1A2. By contrast, attenuation of TCDD-induced IgM suppression required a combination of all three SNPs P517S, R554K, and V570I.

1365 Activation of the Aryl Hydrocarbon Receptor by Categorically Different Ligands Alters the Immune Response to Influenza A Virus Infection

L. A. Boule, G.-B. Jin and B. P. Lawrence. *Environmental Medicine, University of Rochester Medical Center, Rochester, NY.*

The aryl hydrocarbon receptor (AHR) is an environment sensing transcription factor that offers a novel target for strategies to prevent or treat immune-mediated diseases. Exposure to certain AHR ligands alters progression or outcomes in several mouse models of autoimmune or infectious diseases; however, few studies have compared treatment with the same ligands within one *in vivo* disease model. Moreover, for some ligands, it is uncertain whether the immunological consequences are solely AHR-mediated. In this study, we directly compared the effects of 4 AHR ligands on infection with influenza A virus (IAV) in wild-type and Ahr-/- mice: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3,3',4,4',5-pentachlorobiphenyl-126 (PCB126), 2-(1H-Indol-3-ylcarbonyl)-4-thiazolecarboxylic acid methyl ester (ITE), and 6-formylindolo(3,2-b) carbazole (FICZ). TCDD and PCB126 are pollutants with documented human exposure. ITE is being developed as a potential pharmaceutical, and FICZ is a degradation product of tryptophan. We report here that TCDD, PCB126 and ITE, but not FICZ, reduced virus-specific antibody levels and CD8+ T cell responses to IAV. Compared to control mice, TCDD, ITE, and PCB126 reduced the number of Th1 and Tfh cells, whereas FICZ increased their frequency. Th17 cells were not changed after AHR activation by any of the 4 ligands used. Yet, infected mice treated with TCDD, PCB126, ITE, and FICZ all had a greater proportion of Tregs (CD25+Foxp3+CD4+ cells) compared to controls. The specificity of each ligand's actions for the AHR was examined using global Ahr-/- mice, which showed that all of these ligands' effects on T cell responses to IAV infection require Ahr expression. Collectively, this work demonstrates that investigating AHR ligands as therapeutics will require finding a balance between the suppression of immune-mediated pathologies, while maintaining intact and sufficient host defense mechanisms.

1366 Aryl Hydrocarbon Receptor-Mediated Accumulation of Immune Cells in Adipose Tissue of Mice

S. Kado, W. L. W. Chang and C. F. Vogel. *University of California, Davis, CA.*

Lymph nodes are normally surrounded by adipose tissue suggesting a relationship between lymphatic vessels and adipose metabolism. Besides effects on the immune system, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is well known to disturb fat metabolism and to affect gluconeogenesis, glucose transport and adipocyte differentiation. The relationships between the lymphatic system, adipose tissue and immune response/inflammation are quite obvious. Here, we show that activation of the cytosolic aryl hydrocarbon receptor (AhR) by TCDD leads to significant changes of the expression pattern of cytokines and chemokines such as Interleukin (IL)-1β and CXCL5 in epididymal adipose tissue. A clear-cut increase of inflammatory marker genes was also found in other tissues containing lymph nodes, such as mesenteric, perirenal, and the subcutaneous white adipose tissues. Little is known about how changes of cytokine and chemokine expression through activation of AhR will affect chronic inflammatory conditions *in vivo*. We show that the distinct changes of cytokine expression by TCDD in adipose tissue of mice are associated with a rapid accumulation of CD11b+ neutrophils, and recruitment of inflammatory macrophages and dendritic cells in the epididymal, mesenteric, and perirenal adipose tissues of male C57BL6 mice at later time points. Whereas the effect of TCDD on immune cell accumulation was sustained over a period of 6 days, the effect of bacterial endotoxin lipolysaccharide (LPS) on CD11b+ neutrophil recruitment was only transient and returned to a normal level at day 3. Elucidating the role of AhR mediating the rapid and sustained infiltration of immune cells in adipose tissues and their lymph nodes is important to determine the possible involvement in the development of chronic inflammatory diseases.

1367 Impact of AhR Activation on the Phenotype and Function of Bone Marrow Derived Dendritic Cells After Exposure to *Pseudomonas aeruginosa*

S. L. Cole[2], J. M. Kreitinger[2], B. P. Seaver[2], C. A. Beamer[2] and D. M. Shepherd[2]. [1]*Biological Sciences, University of Montana, Missoula, MT and* [2]*Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT.*

Activation of the aryl hydrocarbon receptor (AhR) in dendritic cells (DCs) by the prototypical ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can lead to the induction of a regulatory phenotype and suppressed immune responses to bacterial ligands. Although molecules with a diverse array of chemical scaffolds are known to bind to the AhR and modulate AhR activity, the potential of these ligands to modify the phenotype and function of DCs are currently unknown. Therefore, the goal of the present study was to investigate whether molecules representative of the four families of AhR ligands (e.g. TCDD, benzo(a)pyrene (BaP), leflunomide, and YH439) alter the differentiation and function of bone marrow derived DCs in a ligand-specific and AhR-dependent manner. Similar to TCDD, YH439 induced an immunoregulatory DC phenotype and suppressed immune responses to heat killed Pseudomonas Aeruginosa (HKPA) as measured by changes in the expression profiles of select cell surface markers, cytokines, and transcription factors. In contrast, leflunomide induced almost no change in the expression of differentiation markers and no difference in the response of DCs to HKPA compared to control; whereas BaP showed limited induction of a regulatory DC phenotype and a suppressed response to HKPA. Many, but not all, of the observed changes in the phenotype and function of DCs were dependent on the AhR, as assessed in dendritic cells derived from mice expressing the low affinity Ah receptor (AhRd) and wild-type C57Bl/6 mice. Our findings suggest that induction of an immunoregulatory DC phenotype is AhR-ligand dependent, which implies that environmental contaminants may differentially modulate immunity via changes in the phenotype and function of DCs. This work was supported by NIEHS/NIGMS grant R01 ES013784 (DMS), as well as NIGMS grant P20GM103546. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIEHS, NIGMS or NIH.

1368 Persistent Changes in Dendritic Cell Function Following Developmental Activation of the Aryl Hydrocarbon Receptor

J. L. Meyers[2], B. N. Winans[2], L. Boule[2] and B. P. Lawrence[2]. [1]*Environmental Medicine, University of Rochester, Rochester, NY and* [2]*Microbiology and Immunology, University of Rochester, Rochester, NY.*

The developing immune system is susceptible to environmental insults, which can contribute to altered immune function later in life. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that plays a role in immune system development and function. It binds many exogenous chemicals to which humans are regularly exposed, including dioxins and polychlorinated biphenyls (PCBs). Developmental exposure to a prototype AHR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), deregulates T cell responses to influenza A virus infection later in life. During primary infection, naïve T cells require activation by dendritic cells (DCs); however, the impact of developmental exposure on DCs is poorly understood. We report here that developmental AHR activation alters DC function and distribution later in life. Developmental exposure reduced DC function, as evidenced by a two-fold poorer capacity to stimulate the proliferation and differentiation of naïve virus-specific T cells. While developmental exposure did not change the number of conventional or plasmacytoid DCs in the lung prior to or after infection, there were two-fold fewer DCs in the lung-draining mediastinal lymph nodes (MLN) after infection. A key regulator of DC trafficking from the infected lung to the MLN is chemokine receptor 7 (CCR7). In the MLN there were fewer CCR7+ DCs, and the level of CCR7 expression on DCs was reduced in infected offspring of TCDD exposed dams, compared to DCs from offspring of control dams. Thus, developmental exposure to an AHR ligand diminishes the number of DCs in the MLN following infection, reduces CCR7 levels, and impairs their ability to activate naïve T lymphocytes. Given that DCs regulate T cell responses to many antigens, the discovery that maternal exposure to an AHR ligand perturbs DC function has broad implications for understanding how the early life environment shapes the integrated function of the immune system.

1369 A Computational Approach for Characterizing Subtle Changes in DNA Methylation in CD4+ T Cells

T. Zurlinden[1], K. Gilbert[2] and B. Reisfeld[1]. [1]*Chemical and Biological Engineering, Colorado State University, Fort Collins, CO and* [2]*Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR;* [3]*School of Biomedical Engineering, Colorado State University, Fort Collins, CO.* Sponsor: T. Zurlinden.

Several toxicants are known to cause epigenetic changes, including alterations in DNA methylation, and a growing number of human diseases are now associated with aberrant DNA methylation, including cancer and autoimmune disease. *In vitro* testing for changes in the methylation of individual CpG sites could be useful in screening chemicals for their methylation potential and aid in determining links between observed genotypic changes and adverse health outcomes. Mathematical models can be used to complement such studies by providing a means to interpret the resulting data, compare the extent of DNA methylation across studies, and provide parameters that can be compared among chemicals. Here, we describe the development and use of such a model to characterize time-dependent changes in DNA methylation and methylation variance in naïve and effector/memory CD4+T cells from autoimmune-prone mice. Bisulfite sequencing of CpG sites associated with several functionally important genes was conducted. Results showed that the total methylation variance detected in both naïve and effector/memory CD4+ T cells was substantially lower than that which would have occurred if the methylation were governed by a random process. Moreover, naïve CD4+ T cells, experienced more constraints on methylation variance than effector/memory CD4+ T cells. Overall, these results suggest that DNA methylation patterns in CD4+ T cells are fairly rigid, with natural mechanisms in place that prevent random methylation and demethylation. The model presented in this work has the ability to detect these subtle changes in DNA methylation variance, rather than more unlikely, overt hyper- or hypo-methylation.

1370 Is a "Thresholdable" Carcinogen Still a Delaney Carcinogen?

S. C. Fitzpatrick[1] and A. W. Hayes[2]. [1]*US FDA, College Park, MD and* [2]*Harvard School of Public Health, Andover, MA.*

The 1958 Food Additives Amendment to the Food, Drugs, and Cosmetic Act was a response to concerns about the safety of new food additives. Congressman Delaney believed that there was a connection between increased uses of food chemicals and the rapid increase in reported cancer cases. The Delaney Clause was a provision in the amendment that stated that no food additive shall be deemed to be safe if found to induce cancer when ingested by man or laboratory animals. It applies to all food additives, as well as pesticides used on food crops and veterinary drugs given to food animals. At the time of its enactment, the Delaney Clause may have been a reasonable standard because the comparatively crude scientific detection technology of the time precluded discovery of tiny amounts of chemicals. Additionally, the mechanisms by which chemicals induce cancer were not well understood, and it was assumed that even one molecule of a carcinogen could cause harm. The Delaney Clause has been a source of controversy since its enactment. This Roundtable will debate the relevance of the Delaney Clause to the 21st century regulatory issues that US FDA and US EPA must address. Dr. Keefe from US FDA will present the issues, including the role of Delaney in the current regulatory paradigm. Dr. Hayes will moderate the debate. Some pre-formulated questions will be developed to help guide and stimulate the discussion. Dr. Dourson and Dr. Williams are both highly respected risk assessors. Each debater will present some initial arguments followed by a moderated and lively debate. The audience will be encouraged to participate.

1371 Trichloroethylene Exposure and Development of Fetal Cardiac Malformations: What Do the Data Tell Us About Inhalation Exposures Resulting from Vapor Intrusion and Potential Health Risks to Pregnant Women?

L. C. Haws[1] and B. Sonawane[2]. [1]*ToxStrategies, Inc., Austin, TX and* [2]*US EPA, Washington, DC.*

In 2011, the US EPA developed a Reference Concentration (RfC) for trichloroethylene of 2.0 µg/m3 based on two co-critical studies. One of the co-critical studies reported a dose-dependent increase in fetal cardiac malformations (FCM) in pregnant rats exposed to TCE via drinking water throughout pregnancy. As a result of concerns about this specific end-

point, some EPA regions and states recently issued interim action levels and response recommendations for TCE exposures from vapor intrusion based on accelerated timeframes meant to be protective of the developing fetus during the 3-week period in the first trimester during which the fetal heart develops. These actions have stimulated substantial debate about this particular endpoint within the scientific and regulatory communities, with some arguing that shortcomings of the *in utero* rat study should preclude it from being used as a co-critical study in the TCE inhalation risk assessment, while others argue that the overall weight of the evidence supports the conclusion that FCM is a sensitive adverse endpoint for TCE inhalation exposure, thereby justifying the use of the *in utero* rat study as a co-critical study. This session is intended to stimulate thought-provoking and open dialogue with members of the audience during a moderated panel discussion immediately following the scientific presentations. The moderated panel discussion will incorporate state-of-the-art tools in live audience polling to stimulate discussions. The goal of the roundtable is to identify the specific areas of agreement and/or disagreement and uncertainty regarding the toxicological evidence supporting the association between inhalation exposures to TCE and the potential for FCM and, through discussion, to develop strategies to help bring clarity to these areas.

1372 K–12 Students Flock to ToxTown in San Diego: Results of an SOT K–12 Education Outreach Workshop

V. S. Bhat[2], M. Bourgeois[7], W. Gray[4], J. Sapiro[5], A. Nguyen[1], M. Gwinn[8], A. Slitt[6] and B. Eidemiller[3]. *[1]Kansas State University, Manhattan, KS; [2]NSF International, Ann Arbor, MI; [3]SOT Education Committee, Reston, VA; [4]Southern University, Baton Rouge, LA; [5]University of Arizona, Tucson, AZ; [6]University of Rhode Island, Kingston, RI; [7]University of South Florida, Tampa, FL and [8]US EPA, Washington, DC.*

Just prior to the start of the 2015 Annual Meeting in San Diego, hundreds of K–12 students, teachers, and science enthusiasts visited the ToxTown booth at the annual San Diego Festival of Science and Engineering grand finale event, EXPO Day. Over 20,000 attendees participated in EXPO Day's 130 booths and SOT was well-represented in ToxTown. The Southern California Regional Chapter of SOT (SCCSOT) hosted and funded the booth through a SOT Regional Chapter Strategic Funding grant and through corporate K–12 sponsor funds, with support of the SOT Education Committee and K–12 Education Subcommittee. The students toured ToxTown by first watching a Risk Bites episode on YouTube (www.riskbites.com) produced by 2015 SOT Communications Award winner Dr. Andrew Maynard, who was on hand in the flesh! Students then progressed to a caffeine module learning about dose-response and that caffeine is not always bad for you. Folding Handangos (www.scrubclub.org) to learn about proper hand washing and germs captivated the younger crowd. The favorite activity was Tic-Tac-Tox, in which students spun the cubes to answer toxicology True or False questions hoping to get three-in-a-row to win an SOT-captioned Einstein. The last stop on the tour was a participant survey. Three-quarters of the high school student participants surveyed had "nothing" or "minimal knowledge" of toxicology prior to the event, and 53% agreed that the booth activities helped them "learn about the role of toxicologists in creating a safer and healthier world". The majority of respondents "strongly agreed" that the activities influenced their decision to become scientist or pursue a college degree in toxicology or related health sciences. More than 70% of participants found their "Interaction with a toxicologist at the booth" to be "very informative". Additional itemized survey results will be included in this presentation. Many thanks to SCCSOT and our global slate of SOT members and Annual Meeting attendee volunteers who ensured the event was a tremendous success, as evidenced by the non-stop traffic all day and the multiple social media tweets and "likes"!

1373 K–12 Students Supported by SOT to Attend Central States SOT Regional Chapter Meetings

P. Kasturi[3], U. Apte[3], C. Croutch[2], R. Casillas[2] and A. Nguyen[1]. *[1]Kansas State University, Manhattan, KS; [2]MRIGlobal, Kansas, MO and [3]University of Kansas Medical Center, Kansas, KS.*

Central States SOT (CSSOT) applied for and received a $1,000 grant from SOT to bring local high school students to our regional chapter meeting in the fall of 2014 and 2015. Students from Harmon High School and Washington High School (Kansas City Public Schools) were invited and came to the meeting in Kansas City with their high school administrators. Students began their day by having a "one-on-one" lesson with toxicologists from our group who used K–12 slides to teach them about Toxicology. They then joined the meeting where key note speakers throughout the morning did an outstanding job of including them in their lectures, ensuring that they understood the material of their lectures. Each speaker also provided "words of advice" to the students that were published in the meeting program. Finally, at lunch and during the poster session, the students had the opportunity to meet other Toxicologists attending the meeting. Overall, the feedback from the high school students was very positive and included how much they appreciated the speakers including them in seminars, how interesting the seminars were, and how the activities of the day brought a new understanding of the field of Toxicology to the students. CSSOT attendees were also very enthusiastic about meeting the students and teaching them about toxicology. In continuing efforts to encourage K–12 students to get interested in the science of Toxicology, CSSOT K–12 Representative, Dr. Partha Kasturi with the help of colleagues in the Department of Pharmacology, Toxicology and Therapeutics and the Partnership for Regional Educational Preparation of Kansas City (PREPKC) started a eight week summer internship program at KUMC, Kansas City, KS. Students who attended the CSSOT meeting in 2014 were invited and interviewed for the internship opportunity and three were selected to complete their internships. These students who completed their internship were given an opportunity to present their work to the three faculty who hosted the students and members of the PREPKC. Dr. Kasturi is authoring an update on the students for SOT to publish an article (as part of the Education Committee).

1374 NorCal SOT K–12 Student Outreach: Lessons Learned and Ideas for Future Outreach

D. Zane[1] and T. Parman[2]. *[1]Intarcia Therapeutics, Hayward, CA and [2]SRI International, Menlo Park, CA.*

Education outreach at the K–12 level is an important component in increasing the visibility and perceived importance of toxicology. Scientific experiments in elementary classrooms have been limited due to restricted funding and time. This has led to a concern that there will be a decreased interest in science at a young age, which may translate into a decrease in students entering scientific fields. The Northern California Society of Toxicology (NorCal SOT) has been a vital resource in facilitating student outreach toxicology activities in this region. This abstract will highlight and share its experience in planning and executing successful K–12 outreach activities supported by NorCal SOT over the past several years with practical tips, lessons learned and ideas for future outreach. Past outreach activities includes K–5 hands-on demonstrations and experiments (where students were introduced to Paracelsus, concept of "the dose makes the poison", etc), Annual Girl Scout Science Day Events (where girls performed simple hands-on experiments which were organized in modules covering concepts such as concentrations and dilutions, pH and environment, ADME, etc; organized and implemented by undergraduate and graduate students) and 9–12 Toxicology Exploration and Presentation Program aimed at training the student to conduct basic research in toxicology; these students will have an opportunity to present their work as a poster at NorCal SOT and SOT symposiums. This abstract will also provide insights helpful to those new to educational outreach. Promoting and facilitating K–12 activities has the added potential of greater undergraduate, graduate and postdoctoral engagement and therefore increased opportunities for skill development and instructional background for those trainees considering academic careers. NorCal SOT's goal for K–12 outreach ties directly into the SOT strategic plan of promoting recognition of toxicology and communicating the benefit of toxicology to external audiences, which is vital to maintaining the discipline of toxicology and the pipeline of future toxicologists.

1375 "Tools, Techniques, and Analysis" and "Citizen Science": A New Approach to the Separation of a Single Introductory Laboratory Course into Two New Courses, and the Addition of a Toxicology-Based Exercise to Both

K. E. Stine. *Department of Biology, Auburn University at Montgomery, Montgomery, AL.*

The Department of Biology at Auburn University at Montgomery has moved (based on assessment data) from offering a single Principles of Biology course to offering two separate introductory courses: one primarily for STEM majors and another primarily for non-science majors. While significant (and promising) changes have already been implemented in the classroom in both courses, innovations in the associated laboratories have lagged behind due to limited resources for new equipment and supplies, as well as limited laboratory space (and personnel) for managing two different set-ups each week. Now, however, we are instituting a novel approach which circumvents these limitations, and which other institutions with similar student populations and resource constraints may also find useful. The basis of this is the development of two alternate versions of written laboratory materials which utilize the

same set-up, thus allowing the two courses to share lab space, materials, and effort each week. Both approaches center on the scientific method, but the "Tools, Techniques, and Analysis" materials for STEM majors emphasize building skills in laboratory equipment use and handling of quantitative data, while the "Citizen Science" materials for the non-STEM course emphasize the use of the scientific method to solve problems using everyday materials. We have also recently introduced a new major in Environmental Health and Toxicology, and have taken advantage of this laboratory revision to add a new toxicology-based laboratory exercise (investigating biological membrane damage by chemical and physical agents) to both courses. This poster presents the two versions of this toxicology-based laboratory exercise as a model, first to illustrate how the educational objectives for the two approaches are achieved and assessed in a typical laboratory session; and second, as a demonstration of the use of an introductory laboratory exercise to introduce toxicology to both STEM major and non-majors courses.

1376 Authentic Learning, Collaboration and Student Awareness of Evidence-Based Science

S. Swenson and Y. He. *Sciences, John Jay College, CUNY, New York, NY.* Sponsor: S.-Y. Cheng.

An authentic science learning model through collaborative learning suitable for large urban public colleges with limited resources was proposed and implemented in the course of Instrumental Analysis (science major), Environmental Science (non-science major), and Undergraduate Research at John Jay College, the City University of New York. The goal of this project is to enhance student learning of science concepts and skills by introducing them to real world problems and solutions. Using sustainability-chemistry as an example, this project allows students at different academic levels to work collaboratively on field-based environmental projects to investigate pollutants in Hudson River and other EPA Superfund sites in New York City, establish close communication among participants and mentors, and develop positive attitudes towards science learning, and cultivate social awareness. This research is a result of National Science Foundation funding: NSF TUES DUE_1245314

1377 Scaffolding Assessment of Forensic Toxicology Program

S.-Y. Cheng and S. Swenson. *Sciences, John Jay College of Criminal Justice, New YORK, NY.*

Scaffolding is considered to be an important element of effective teaching and learning. Previous research has shown that hierarchical organization of problem-solving strategies has positive outcomes for student learning and that helping students organize their knowledge is as important as learning the knowledge itself. Organizing hierarchical knowledge is a way to scaffold information and in our study we created a treatment for scaffolding laboratory knowledge and skills and assessed these scaffolding strategies. This presentation reports the findings of a small-scale initiative undertaken in Forensic Toxicology program of John Jay College to investigate how scaffolding the curricula may affect student outcomes. Students from General Chemistry (CHE104, Freshman year, N=20), Quantitative Analysis (CHE220, Sophomore year, N=21), Instrumental Analysis (CHE321, Junior year, N=10), and Analytical Toxicology (TOX416, Senior year, N=20) were participated in this assessment. Only one of Department learning goals, which is Practical Skills, was assessed. A laboratory report was used as an assessment tool. Students were provided with a clear requirement of laboratory writing guideline. The Assessment tool, rubrics modified from AACU, examined 3 different aspects of student learning about Practical Skills—Inquiry and Analysis; Quantitative Literacy; and Problem Solving. Two faculty members evaluated those reports independently. The results were encouraging. For Inquiry and Analysis aspect, students' performance in these 4 courses met expectations, except TOX416 in one of the sub-areas (Limitation and Implications). For Quantitative Literacy, students' performance in these 4 courses met expectations. For Problem Solving, students' performance in these 4 courses met expectations, except TOX416 in one of the sub-areas (Evaluate outcomes relative to the problem). One interesting finding is that even though CHE220 students' average performance was above satisfactory, the distribution of students' performance was much wider than in other courses. More data will be collected for CHE220 to investigate this wide distribution.

1378 An Inquiry-Based Cultural Eutrophication Lab: Experimental Design, Data Analysis, and Conclusions

M. Pomeroy-Black and M. D. Yates. *LaGrange College, LaGrange, GA.* Sponsor: M. Pomeroy-Black.

LaGrange College is situated near West Point Lake, which experiences periods of cultural eutrophication due to runoff and discharge into the Chattahoochee River from Atlanta. To help students understand the implications of cultural eutrophication in our environment, an experiment was designed by which the students would mimic these events. Students first completed a guided literature review about the sources and effects of cultural eutrophication. They then designed an experiment in which fertilizer was added to two closed bodies of water. Students examined either a concentration-dependent response or a time-dependent response between the two bodies of water for one week. The bodies of water were placed under a 24-hour light source over the course of the week. There were two endpoints in their experiment: turbidity and algal production. Every other day, students measured turbidity using a light meter. At the end of the week, students used an ethanol extraction method to separate photosynthetic pigments from algae in each treatment. Students measured chlorophyll A concentration using light spectroscopy as an estimate of algal production. Chlorophyll A data were analyzed using a t-test. Finally, students prepared a research report using an integrated approach, presenting the Chlorophyll A data as a bar graph and turbidity values as a line graph. This lab can also be easily adapted for a non-majors introductory course by simplifying it using only turbidity data.

1379 What's in Your Water? Infusing Environmental Toxicology into a Non-Majors Informatics Course

C. P. Curran[2], J. Moses[2] and M. Day[2]. [1]*Biological Sciences, Northern Kentucky University, Highland Heights, KY and* [2]*Communication, Northern Kentucky University, Highland Heights, KY.*

The Northern Kentucky University (NKU) Center for Environmental Education sponsored a grant competition to encourage faculty to incorporate environmental information into non-science courses. Our proposal to combine the expertise of faculty and students in environmental science and communication was successful and led to the development of an Environmental Informatics module for the general education course Principles of Informatics in Fall 2014. The over-arching goal was to help students sift fact from fiction in media reports and on Internet sites covering environmental issues, so they can be better informed citizens. Therefore, they reviewed actual news articles about water quality issues, including the Toledo toxic algae water crisis and the 2014 Elk River, West Virginia chemical spill. Then, they learned how to use a water quality app developed at NKU to determine if area streams were polluted or in good health, learned about the role of citizen-scientists in environmental monitoring, and discovered how the EPA's Toxic Release Inventory can be used to identify major polluters in the Greater Cincinnati region. Finally, they tackled a fictional spill on a local creek pitting an environmental activist group against a local corporation. Working in the role of environmental reporters, the students were able to interview a local biologist as an independent expert before writing news headlines about the event. Pre-and post-testing was done to assess environmental knowledge. Students showed greatly increased awareness about environmental issues after completing the module and overwhelmingly supported the use of citizen-scientists for environmental monitoring.

1380 Women in Science (WIS) STEM Program for 6th and 7th Grade Girls Features Toxicology Education

S. Ford[2], D. Hardej[2], E. Megehee[2] and M. Nitopi[2]. [1]*Chemistry Department, St. John's University, Queens, NY and* [2]*Grants and Sponsored Research, St. John's University, Queens, NY;* [3]*Pharmaceutical Sciences, St. John's University, Queens, NY.*

The Women in Science Association at St. John's University developed a STEM program for forty 6th–7th grade girls in the Queens, New York vicinity. The goal of the program was to introduce STEM related topics to the female participants. The program was conducted four Saturdays from Feb through April and featured faculty presentations on STEM related topics including two sessions on toxicology. St. John's WIS students assisted in these classes and were role models for the girls. The first session presented general information in toxicology such as terminology, history and basic concepts. Hands-on demonstrations were conducted on dose response and signal words found on household

products. Students were given a pre- and post-assessment to determine familiarity with the concepts prior to the session and if they retained the information after the session was given (40% pretest versus 80% posttest). Environmental toxicology was featured in the second session exploring the presence of toxicants in the environment and included discussion of toxic metals and industrial chemicals. The girls participated in an experiment called "No Water off a Ducks Back" in which they examined feathers and eggs exposed to water containing oil. The participants recorded their findings, discussed them and made some conclusions on possible effects of oil spills on wildlife. Other demonstrations related to chemistry, solar energy, genes and using maps. Participants of the program were selected based on teacher recommendations and willingness to participate in the program. Students were asked to complete a survey to discuss their experiences, and to choose the session they felt had the most impact on their learning. All students reported that they learned a great deal from sessions of the program. Toxicology was chosen by many of the students as a session that felt had the most impact on their learning. This program was supported by a grant from the Patrina Foundation.

PS 1381 Toxicarnival: A Toxicology Awareness Program Using Carnival Games to Teach Basic Toxicology Concepts

D. Hardej and B. Kistinger. *Pharmaceutical Sciences, St. John's University/Mid-Atlantic SOT, Queens, NY.*

ToxiCarnival, is an educational program that was developed by the MASOT Education and Outreach Committee to teach basic toxicology concepts to undergraduate students using carnival games. The program was first developed and carried out on October 31, 2014 on St. John's University Queens, NY campus as a pilot program in order to test the concept on undergraduate students and to be used in the future for younger age groups. The program was constructed with a carnival theme and carnival games were given names related to toxicology such as Toxic Duck Match, Wheel of Poison, Deadly Disk Drop, and The Circle of Death Ring Toss. Visitors were required to answer toxicology questions in order to play the games and win small prizes. A rough assessment of the questions was made by tallying correct and incorrect responses to each of the questions. The Toxicology Club of St. John's University (Tau Omega Chi) worked in conjunction with MASOT's Education and Outreach members to carry out the event. In this regard, ToxiCarnival served the purpose of providing toxicology education and outreach to all majors of the university community and also reinforced essential concepts in the toxicology majors that were administering the questions. Questions were developed by Tau Omega Chi and the MASOT Education and Outreach Committee members. All questions were then reviewed by the Committee to determine if they were appropriate and accurate. University students of a variety of majors visited the ToxiCarnival and were very engaged in the questions and activities presented at the event which was conducted again in fall 2015 for college students and adapted for a STEM event as a science carnival on October 31, 2015.

PS 1382 Translating Toxicology for the General Public: Novel Approaches for Reducing Traffic-Related Air Pollution

M. Lyons[3], K. Ollier[3], K. Brown[3], A. Zekic[3], J. Hockenbury[3], C. Marini[3], Z. Hart[3] and C. P. Curran[3]. [1]*Biological Sciences, Northern Kentucky University, Highland Heights, KY;* [2]*Business Informatics, Northern Kentucky University, Highland Heights, KY and* [3]*Communication, Northern Kentucky University, Highland Heights, KY.*

Vehicle emissions are the second leading contributor to greenhouse gas emissions with passenger cars and trucks accounting for the majority of emissions. The adverse health effects of traffic-related air pollution (TRAP) are well known. In addition to increasing the risk of asthma and other respiratory disorders, air pollutants can decrease IQ and increase the risk of cardiovascular disease, cancer, birth defects, and allergies. Water quality is also affected by heavy vehicular traffic. For example, peak stormwater runoff from Cincinnati interstates contains heavy metal concentrations in excess of Ohio EPA water quality standards. Unfortunately, commuter habits are hard to change. Up to 70% of commuters express an interest in using transit, but less than 10% regularly do so. To tackle this problem, Northern Kentucky University formed a transdisciplinary team under the US EPA's P3 (People, Prosperity and the Planet) program. Environmental scientists worked with students in computer science, public relations, graphic design and business informatics to explore commuter behavior in an effort to promote green transportation options including carpooling, transit use, and cycling or walking for short trips. A "Choose Green" awareness campaign occurred during the 2014–2015 academic year with three key messages. Usage of TANK buses was up in the first three months after the awareness campaign began. Web surveys indicated the most motivating message was overwhelmingly "Save Money" with a 67% response rate compared with 20% for "Save the Planet" and only 13% for "Get Healthy." Tabling events included a 3-dimensional display of the effect of single-occupancy vehicles v commuting, a traffic trivia game, and a display showing the pollution generated by one mile of driving converted to the number of cigarettes smoked. Supported by US EPA SU835703 and ES020053.

PS 1383 Introducing Toxicology Concepts in General Biology Courses Through Inquiry Based Modules

T. M. Borrás-Pacheco, H. M. Orozco-Mercado, W. Torres-Rivera and L. B. Mendez. *School of Science and Technology, Universidad del Este, Carolina, PR.*

Inquiry based teaching laboratory exercise modules are used as a strategy in science courses to enhance critical thinking, formulate hypotheses, and acquire problem-solving skills among others competencies. In teaching institutions with limited research capabilities, most undergraduate (UG) students do not have the opportunity to participate in research projects. The main goal of this project was to design inquiry lab modules to introduce toxicology concepts through research activities in the General Biology course. The objective of this project is to develop research-oriented activities to assess the adverse health effects of air pollutants. The modules were developed in conjunction with UG students who have previously taken the course. The following concepts were incorporated in the modules: air quality index, bioindicators, model organisms, phylogeny and bioinformatics, toxicity and dose-response relationships. To introduce the air quality index concept, strips for passive sampling of particulate matter (PM) and ozone were used. Lichens were used as bioindicators to assess the relative amount of metals present in PM. Planarians were used as a model organism of mucociliary impairment. Bioinformatics tools were used to generate phylogenetic trees to determine genetic relatedness of invertebrate and vertebrate cilia. To assess toxicity and dose-response relationships, planarians were exposed to increasing concentrations of PM and their locomotion was determined before and after exposure. Determination of the air quality index and data obtained from the lichens provided the basis to formulate hypotheses to be tested *in vivo* with the planarians. While the use of bioinformatics tools allowed students to understand the usefulness of model organisms in toxicology. The feedback from UG students who had previously taken the course permitted the tailoring of modules to the course syllabus, the time allotted for the laboratories, and to incorporate their suggestions regarding its applications to other courses. In the future we will implement the modules in the General Biology course and evaluate student attitudes regarding the experience.

PS 1384 Incorporating Toxicology in a Public Health Summer Institute–A Golden Opportunity to Reach High School Students

R. Salazar and M. Bourgeois. *Environmental and Occupational Health, University of South Florida College of Public Health, Tampa, FL.*

Most toxicology programs are housed in colleges of medicine, pharmacology and veterinary science. The University of South Florida Toxicology program is one of the few located in a college of public health. The USF COPH toxicology program is part of the Environmental and Occupational Health Department; courses here stress 'big picture' approaches to the EOH problems of today. The practice of public health focuses on communities rather than individuals and stresses prevention over cure. Every summer, USF COPH sponsors several sessions of a 'Summer Institute in Public Health'. Students begin the week with a brief overview of the Institute agenda and a campus tour. With a bit of planning, a motivated professor can act as both chaperone and 'stealth educator'. For example, the trip to campus wellness includes a Q&A on binge drinking and substance abuse. This is an ideal segue into the mechanisms of intoxication, metabolism and drug testing. Walking back to the classroom allows the instructor to connect toxicology to the environment, human health, risk assessment and policy development via Socratic questioning. Faculty and graduate students from the 5 departments visit with the campers over the course of the week. Students also have the opportunity to tour laboratories on and off main campus. It can be challenging to keep students engaged so lectures are brief and combined with active learning exercises that include CPR instruction, fire suppression, food safety, disaster response, epidemic surveillance and industrial hygiene. Instructors may crosswalk the activity back to their own discipline but these activities are also opportunities to integrate basic toxicology concepts into environmental health, epidemiology, biostatistics and global health. For example, students are taught to streak plate bacteria after their food microbiology lecture. They are also

given petri dishes with simulated colonies of *Escherichia coli* O157:H7. Now they can also learn about the enterovirulence of the endotoxin in addition to prevention strategies and treatment modalities. These week long camps are designed to acquaint motivated students with the theory and practice of public health. Based on the round table discussions, individual reflections and surveys on day 5, they are also a surprisingly effective way to introduce students to the science of toxicology and its often unnoticed role in their world.

1385 Inspiring the Next Generation to the World of Toxicology: Successful K–12 Outreaches

J. E. Cañas-Carrell, L. Ponnusamy, K. Wooten, E. Reátegui-Zirena and K. Thompson. *Environmental Toxicology, The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX.*

Science is fun for school children but what is toxicology and what toxicologist do as a career are the questions students are not aware of. Here comes the importance of toxicology outreach to introduce, inspire and engage school children to the world of toxicology. Lone Star chapter of Society of Toxicology K–12 outreach members/volunteers have accomplished successful toxicology summer camps conducted by Llano Estacado chapter of the Society of Environmental Toxicology and Chemistry at Texas Tech University, Lubbock, Texas. The summer camp named Science It's a Girl Thing (SIGT) held in June 2015 had a group of 20 middle school girl students. Another summer camp called Shake Hand with Your Future (SHWF) held in July 2015 had a session of 14 elementary and middle school students and another session of eight students from high schools in and around Lubbock, Texas. All of these camps were residential summer camps centered on introducing the underrepresented school students into collegiate experience by providing strong role models in STEM. The four days summer camp on environmental toxicology session consisted of an interactive introduction to toxicology as a science along with presentations and experiments on various toxicological hands-on training activities. All three sessions were started with brainstorming activity to define toxin, toxicology, environment, and environmental health and concepts on applying basic science concepts into toxicology. Hands on experiments included analysis on toxicity testing using dose -response curve, DNA extraction, threshold limit taste experiment, and lake contamination. In addition, success stories of toxicologists as well as women scientists were presented. The main focus of the summer camp was to introduce the different sections of toxicology (such as analytical, environmental, marine and molecular toxicology along with activities for each section) and wind up with toxicology crosswords and jeopardy. Participation of some of the students in both SIGT and SHWF camps demonstrates the inspiration they received. The successful outcomes of these toxicology outreaches were not only introduced the next generation to toxicology but also inspired the underrepresented students including more girls into STEM in general.

1386 The Toxicology Education Foundation: Taking the Message Public

P. Wexler. *National Library of Medicine, Bethesda, MD.*

Established in 1990, the Toxicology Education Foundation (TEF) is devoted to educating the public about the potential hazards of chemicals and other agents to which we are exposed throughout our daily lives. As such, it develops resources to convey basic toxicological principles in a scientifically accurate and easy to understand manner. Its award winning video, Is it Safe?, available in English, Spanish, and Japanese, has offered diverse public audiences the opportunity to make informed decisions about the extent of risk and/or relative safety of chemicals. More recently produced, its short format YouTube video, Toxicity Today," provides an entertaining look at the basics of dose, exposure, hazard and risk. TEF's website (http://toxedfounation.org) underwent a major renovation in 2015. In addition to a more coherent design, vibrant graphics, and improved navigation, a major feature is the inclusion of Tox Topics, presenting capsule summaries of important toxicological issues and including, for each, selective links to further sources of information. Stringent selection criteria assure linkages to quality sites. Several of the potpourri of topics already completed in this ongoing series are: hazard vs. risk, cosmetics safety, toxicity testing, green chemistry, basics of dose and exposure, genetically modified organisms, pool chemical safety, botulinum toxin and botox, water intoxication, green chemistry, radiation, and occupational safety and health. Tox Quick Links, connects users with Web sites representing glossaries, tutorials, and other instructional materials; databases; governmental organizations; and professional societies and other groups. Although TEF's focus is on the public, it has also contributed curriculum materials in toxicology to school age students and teachers, and offers an award to graduate students majoring in reproductive toxicology. TEF's international roster of Trustees consists of prominent toxicologists with a strong interest in demonstrating to diverse communities the relevance of toxicology to public health and safety. TEF is the rare group that takes the message of toxicology directly to the public.

1387 MUS81-EME1 as a Novel Anti-Cancer Therapeutic Target

S. Mukherjee[1], K.-H. Ang[3], J. Chau[1], H. Yakubi[1], M. Arkin[3] and W.-D. Heyer[1,2]. [1]*Microbiology and Molecular Genetics, University of California, Davis, Davis, CA;* [2]*Molecular Cellular Biology, University of California Davis, Davis, CA and* [3]*Small Molecule Discovery Center, University of California, San Francisco, San Francisco, CA.*

Traditional DNA damage-based anti-cancer therapies target the enhanced rates of replication of cancer cells, but are accompanied by adverse affects to normal cells. A novel therapeutic approach is to exploit the genetic differences between cancer cells and normal cells by targeting MUS81-EME1, an enzyme involved in DNA damage repair by homologous recombination (HR). Importantly, MUS81-EME1 becomes essential in 10–15% of cancers that rely on Alternative Lengthening of Telomeres (ALT). In order to develop small molecule inhibitors that are feasible for clinical use, milligrams full-length human MUS81-EME1 has been purified from Sf9 cells that over-express MBP-MUS81/GST-EME1. The enzyme is catalytically active and has similar kinetics to the well-studied yeast homolog Mus81-Mms4, with KM = 25 nM and kcat = 1.5 min-1 for preferred nicked DNA substrates that represent intermediates that arise in HR. An initial pilot screen of 2,217 compounds from the MicroSource library with a Förster Resonance Energy Transfer (FRET)-based nuclease assay reveals a 3% hit rate. 8 hits have been re-confirmed in concentration-response assays to have IC50s as low as 30 nM. A primary screen of 150,000 novel compounds with the FRET-based assay is in progress. The hits from the pilot and primary screen will be confirmed in the established secondary cell-based high throughput viability assay where MUS81-EME1 inhibition in ALT cancer cells leads to up to 72% decrease in viability compared to no inhibition controls. Lead inhibitors may serve as adjuvant therapy with existing chemotherapy or as potential anti-cancer drugs in telomerase-negative/ALT-positive cancers where MUS81 is essential. This work represents a study of targeted drug discovery that exploits tumor dependence on HR.

1388 The Mutagenic Potential of *Aloe vera* Extracts in Mouse Lymphoma Assay

X. Guo, S. Dial, N. Mei and M. Moore. *NCTR/US FDA, Jefferson, AR.*

Aloe vera has long been used as a traditional medicine for the treatment of diverse disorders and ailments. Now it is used as an ingredient in a variety of food and cosmetic products. However, clear evidence of carcinogenic activity of *Aloe vera* whole leaf extract in rodents raises safety concerns for human exposure. To provide insight into the identity and mechanisms associated with mutagenic components of the *Aloe vera* extracts, the mutagenicity of *Aloe vera* whole leaf extract (WLE) and *Aloe vera* decolorized whole leaf extract (WLD) were evaluated using the mouse lymphoma assay. The WLD extract was obtained by subjecting WLE to activated carbon-adsorption. HPLC analysis indicated that the decolorization process removed a large number of components from the WLE extract, including anthraquinones. Both WLE and WLD extracts showed cytotoxic and mutagenic effects in mouse lymphoma cells but at different concentration ranges, and WLD induced about 3-fold higher levels of intracellular reactive oxygen species (ROS) than WLE. Molecular analysis of mutant colonies from cells treated with WLE and WLD revealed that the primary type of damage from both treatments was largely due to chromosome mutations (deletions and/or mitotic recombination). The fact that the samples were mutagenic at different concentrations suggests that while some mutagenic components of WLE were removed by activated carbon filtration, components with pro-oxidant activity and mutagenic activity remained. The results demonstrate the utility of the mouse lymphoma assay as a tool to characterize the mutagenic activity of fractionated complex botanical mixtures to identify bioactive components. The results also cast doubt on the hypothesis that the carcinogenicity of WLE is primarily due to the presence of anthraquinones and that eliminating these chemicals will eliminate hazard attributable to genotoxicity.

1389 Analysis of Inherited Germline Mutations of ENU-Treated Mice by Whole Exome Sequencing

K. Masumura[3], N. Toyoda-Hokaiwado[3], A. Ukai[3], Y. Gondo[2], M. Honma[3] and T. Nohmi[1,3]. [1]*Biological Safety Research Center, NIHS, Tokyo, Japan;* [2]*RIKEN BioResource Center, Ibaraki, Japan and* [3]*Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan.* Sponsor: A. Nishikawa.

Germline mutations are an important topic in toxicology because they are heritable and some genetic changes may cause health disadvantages in the next generation. Recent advances in high-throughput sequencing technologies will expand the use of direct sequencing for mutation analysis. To investigate inherited germline mutations, N-ethyl-N-nitrosourea (ENU)-induced trans-generational mutations were analyzed by whole exome sequencing. Male gpt delta mice were treated with ENU (85 mg/kg i.p., weekly x 2). Ten weeks after the last treatment, the mice were mated with untreated females and offspring were obtained. At 20 weeks after the treatment, gpt mutant frequencies (MFs) in the sperm of the ENU-treated mice increased 22-fold higher than those of the untreated control. Genomic DNA of one ENU-treated and one control families were applied to whole exome sequencing analysis. Single nucleotide variations were compared between parents and offspring and *de novo* mutations in the offspring were detected. In the ENU-treated group, total 148 mutation candidates were detected in 4 children and 82% (123/148) was confirmed as true by Sanger sequencing. In the control group, 12 mutation candidates were detected and 25% (3/12) was confirmed. The confirmed germline mutation frequency in the offspring of the ENU-treated father increased 17-fold higher than that of the control group, it could be comparable fold-increase with the gpt MFs. *De novo* mutation spectrum in the next generation showed characteristics of ENU-induced mutations such as base substitutions at A:T bps. Especially, A:T to G:C (42%) and A:T to T:A (27%) were predominantly observed by exome sequencing. These results suggested that the direct sequencing analysis could be a useful tool to investigate inherited germline mutations and evaluate mutagenic effects of genotoxins in the next generation.

1390 Bisphenol A Alters Cellular Microenvironment to Promote Survival After Oxidative Stress

N. R. Gassman[1], E. Coskun[2], P. Jaruga[2], M. Dizdaroglu[2] and S. H. Wilson[3]. [1]*University of South Alabama Mitchell Cancer Institute, Mobile, AL;* [2]*Biomolecular Measurement Division, National Institute of Standards and Technology, Gaithersburg, MD and* [3]*Genome Integrity and Structural Biology Laboratory, NIEHS/NIH, Research Triangle Park, NC.*

Exposure to the environmental endocrine disrupting chemical bisphenol A (BPA) has been reported to alter global gene expression, induce epigenetic modifications, and interfere with the complex regulatory networks of cells. In addition to these reprogramming events, we have demonstrated that BPA exposure generates reactive oxygen species and promotes cellular survival when co-exposed with dietary oxidizing agent potassium bromate (KBrO3). In this study, we aimed to determine the cellular microenvironment changes induced by BPA co-exposure that promoted cell survival and determine if these changes were unique to co-exposure. Ku70-deficient cells were exposed to BPA, KBrO3, and co-exposed with both agents. 4 and 24 h post-damage initiation, we performed whole genome microarray analysis and evaluated chromatin structure, glutathione content, and intracellular pH. 4 h post-damage initiation BPA co-exposure suppressed DNA repair by condensing chromatin and reducing transcription of DNA repair proteins. BPA also stabilized the intracellular pH change observed after KBrO3 only treatment. 24 h post-damage initiation, BPA exposed cells showed less condensed chromatin; their intracellular glutathione was slightly depleted; intracellular pH was reduced, while KBrO3 alone showed an increase in pH; and significant up-regulation in DNA repair proteins was observed only for the co-exposure condition. These results support an induction of an adaptive response due to BPA co-exposure that results in the delayed repair of oxidatively-induced DNA lesions. Further work is required to understand the long-term consequences of this delayed repair; however, this study demonstrates that BPA exposure significantly alters the cellular microenvironment to promote survival.

1391 The Non-Narcotic Antitussive Benzonatate Is Not Mutagenic or Genotoxic in *In Vitro* and *In Vivo* Assays

K. Dobo[2], W. Madraymootoo[1], V. Wagner[1] and S. Teo[3]. [1]*BioReliance, Rockville, MD;* [2]*Pfizer, Groton, CT and* [3]*Pfizer, Madison, NJ.* Sponsor: S. Teo.

Benzonatate (TESSALON®) is a non-narcotic, peripherally acting, oral prescription antitussive for the symptomatic relief of cough. It is an ester type anesthetic structurally related to procaine and is thought to inhibit cough by anesthetizing pulmonary stretch receptors which mediate the cough reflex. It is rapidly metabolized by body esterases to the major metabolite 4-(butylamino)benzoic acid (BBA). A battery of genotoxicity studies were performed on B and BBA. Bacterial reverse mutation/ Ames assays used S. typhimurium TA98, 100, 1535, 1537; E. coli WP2 uvrA tester strains, ± Aroclor-induced rat liver S9. In initial toxicity-mutation assays, the maximum dose was 5000 µg/plate (1.5 - 5000 µg/plate). There was no precipitate. Toxicity was observed with BBA under some conditions. In confirmatory assays, no mutagenic responses were observed at up to 5000 µg/plate (± S9). Both B and BBA were therefore negative for mutagenicity. The *in vitro* mammalian chromosome aberration test assessed clastogenic potential using human peripheral blood lymphocytes. Peripheral blood lymphocytes were treated for 4, 20 hr -S9 and 4 hr +S9 up to 1930 µg/mL. All cells were harvested 20 hr after treatment. Benzonatate did not induce structural or numerical chromosome aberrations ±S9. BBA induced aberrations at 4 hr ±S9 but only at cytotoxic and precipitating doses. In the 20-hr exposure -S9, no aberrations were noted at comparable cytotoxicity without precipitation. BBA therefore did not induce structural and numerical chromosome aberrations ±S9 activation. The incidence of micronucleated polychromatic erythrocytes (MPCEs) was determined in bone marrow of male and female CByB6F1 mice orally dosed with up to 250 mg/kg B for 28 days. Cyclophosphamide positive control was dosed once at 50 mg/kg. Femoral bone marrow was collected, smeared, stained and 2000 polychromatic erythrocytes (PCEs)/animal were evaluated for MPCEs. No reductions in the PCEs/Erythrocytes ratio were observed in B groups relative to Control indicating that it was not cytotoxic. No statistically significant increase in the incidence of MPCEs was observed. Benzonatate was therefore not genotoxic. In summary, B and BBA were not mutagenic or genotoxic.

1392 The New Generation of the DEL Assay Detecting "Non-Mutagenic" Carcinogens

R. Schiestl, L. Parfenova, D. Nguyen and V. Kutepova. *Pathology and Environmental Health Sciences, UCLA, Los Angeles, CA.* Sponsor: R. Schiestl.

Genetic instability is a hallmark of carcinogenesis. Furthermore, cells from patients carrying mutations conferring cancer prone phenotypes show a higher level of genetic instability, including DNA deletions. In fact, the original yeast-based DEL (deletion) Assay with 100 chemicals shows an accuracy of 92% to detect carcinogens as compared to 62% detected with the Ames Assay. DEL events in all three formats are inducible by a wide variety of carcinogens including carcinogens that are negative in many other short-term tests. Here we introduce the next generation DEL assay: a novel dual-read out *Saccharomyces cerevisiae* screen DEL-Lac. DEL-Lac simultaneously assesses the compound's genotoxicity and cytotoxicity properties. During an exposure to a genotoxic event the sequence is excised and the lacZ gene recombines to a functional beta-galactosidase genotype that with an addition of the X-Gal substrate produces a strongly positive indigo (blue) product. What is the most important advance is that we can determine the effect of target gene expression on genotoxicity. Surprisingly and most importantly we found that the Ames negative carcinogens that were weakly positive in our standard His reversion assay, are 10 fold more potent in expressed DNA. This makes the new version of the DEL assay even more useful and makes sure that the Ames negative carcinogens will be positive even in the high throughput version. DEL-Lac is a rapid and economical way to screen large chemical libraries for toxicity and potential carcinogenicity properties.

1393 Red Blood Cell Pig-a Assay and Pigret Assay in Rats with Pyrene

I. Yoshida, A. Matsumoto, Y. Sakai, Y. Harada and T. Hashizume. *Drug Safety Research Laboratries, Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, Fujisawa, Kanagawa, Japan*. Sponsor: M. Friedman.

A new *in vivo* gene mutation assay has been developed based on the phosphatidylinositol glycan anchor biosynthesis, Class A gene (Pig-a in rodents) as an endogenous reporter. Using this Pig-a assay, the *in vivo* mutagenicity of the pyrene (Pyr) was investigated after a single dose on the red blood cell (RBC Pig-a assay) and on reticulocytes (PIGRET) in rats. Seven-week old male rats were orally dosed once with Pyr at 500, 1000 and 2000 mg/kg or ethylnitrosourea (ENU) at 10 and 40 mg/kg as a positive control. Because 2 out of 6 animals at 2000 mg/kg of Aza died 3 days after the dosing, this dose group was excluded for analyses. The frequencies of Pig-a mutants in RBCs and reticulocytes were evaluated once a week for 4 weeks after the treatment. With a single exposure to ENU, the frequencies of Pig-a mutants in both RBCs and reticulocytes increased in a time- and dose-dependent manner. In contrast, no statistically significant effect was observed in rats at any dose of Pyr in the RBC Pig-a assay and the PIGRET assay. Based on the present results, the mutagenicity of Pyr is concluded to be negative after single oral administration in rats.

1394 Validation of the Neutral Red Uptake Assay in BALB/c 3T3 Cells

C. S. Farabaugh, S. B. Hurtado and M. M. Wells. *Genetic and In Vitro Toxicology, WIL Research, Skokie, IL*.

WIL Research has established methods for the Neutral Red Uptake Assay (NRU) using BALB/c 3T3 cells in accordance with the OECD Test Guideline 129. The NRU Assay is used to generate *in vitro* cytotoxicity data for the determination of starting doses for rodent acute oral toxicity tests, reducing animal use. Briefly, BALB/c 3T3 cells are exposed to a range of test article concentrations in a 96-well plate for 48 hours. At the end of exposure, the test article is removed and neutral red (NR) is added to the cells for an incubation of 3-hours. Viable cells will incorporate and bind the NR, such that cytotoxicity is expressed as a concentration-dependent reduction in the uptake of NR. Following incubation, the NR media is removed, cells are washed, and a desorb media is added to extract the NR from the cells. Absorption is measured at 540 nm. The data generated is fitted with a 4-parameter curve, and the IC50, the concentration at which 50% cytotoxicity is observed, is calculated. Preliminary method development runs conducted with the positive control, sodium lauryl sulfate (SLS) have yielded a negative control absorption of <0.05, a vehicle control absorption of approximately 0.2 0.4, and an average IC50 for SLS of 20.17 ± 3.29 µg/mL. The preliminary results align with published values, and indicate that the method under validation is an appropriate method for the estimation of cytotoxicity using the *In Vitro* NRU Assay.

1395 Propargite Technical: *In Vivo* Mutation Assay at the cII Locus in Big Blue® Transgenic F344 Rats

C. Koenig[1], R. Young[2], R. Kulkarni[2], M. McKeon[2], R. Russell[2], E. Freeman[4] and B. Gollapudi[4]. [1]*Arysta LifeScience, Waterbury, CT;* [2]*BioReliance Corporation, Rockville, MD;* [3]*Center for Chemical Regulation and Food Safety, Exponent, Midland, MI and* [4]*Center for Toxicology and Mechanistic Biology, Exponent, Midland, MI.* Sponsor: C. Koenig.

The purpose of the present investigation was to evaluate the *in vivo* genotoxic potential of propargite technical (2-(p-tert butylphenoxy) cyclohexyl 2-propynyl sulfite, CAS# 2312-35-8), an organosulfur acaricide. In a two year cancer bioassay, propargite produced tumors in the jejunum of rats. These tumors were believed to be the result of prolonged localized irritation and not due to a genotoxic mode of action. To confirm this hypothesis, male Big Blue® F344 rats were administered propargite via feed for 28 days ad libitum at dose levels of 0, 80, 400 and 800 ppm (tumorigenic dose) using a study design compliant with OECD Test Guideline 488 - Transgenic Rodent Mutation Assays. On Day 26 peripheral blood was collected for evaluation of micronucleated reticulocytes by flow cytometry. After 28 days of exposure, animals were switched to untreated feed for three additional days. On Day 31 animals were sacrificed and the liver and jejunum (tumor target tissue) were processed for determination of mutant frequency at the cII locus of the shuttle vector. Following exposure to propargite there was no statistically elevated mutant frequency at the cII locus in either the liver or jejunum: group mean mutant frequencies in treated rats were 29.7 ± 8.2 x 10-6, 33.5 ± 10.2 x 10-6, 38.8 ± 15.7 x 10-6 and 38.2 ± 11.4 x 10-6 in liver, and 45.4 ± 10.3 x 10-6, 36.8 ± 12.3 x 10-6, 40.9 ± 11.0 x 10-6 and 27.7 ± 9.1 x 10-6 in jejunum, for treatment groups 0, 80, 400, and 800 ppm, respectively. Vehicle control mutant frequencies were comparable to historical values. DNA from the liver and jejunum of Big Blue rats exposed to the positive control (40 mg/kg/day N-ethyl-N-nitrosourea) for 3 days and necropsied on day 31 yielded significantly elevated mutant frequencies of 128.9 ± 35.0 x 10-6 in liver and 209.8 ± 54.4 x 10-6 in jejunum, demonstrating the sensitivity of the assay to detect mutagenesis in the study tissues. There were no significant increases in micronucleated reticulocytes in peripheral blood samples collected on Day 26 in treated animals when compared to controls. The negative results for mutagenicity and cytogenetic damage in this study provide further support for a non-genotoxic mode of action in the etiology of jejunum tumors seen in the propargite two year cancer bioassay.

1396 The Development of a Modified Comet Assay for Assessment of Global and Region-Specific DNA Methylation Status

T. A. Townsend, M. E. Bishop, S. D. Shelton and M. G. Manjanatha. *US FDA, NCTR/DGMT, Jefferson, AR*.

Assessing DNA damage and epigenetic modifications, including DNA methylation, is critical in predicting carcinogenicity of pharmacological and biological agents. The single cell gel electrophoresis (Comet) assay is validated for regulatory use, with an OECD Test Guideline (TG489) approved in 2014 for conducting the *in vivo* Comet assay. Here, we utilized the methylation-dependent restriction endonuclease, McrBC, to develop a modified alkaline Comet assay that allows the use of a single platform to evaluate genotoxicity, global and region-specific DNA methylation status of single cells under various conditions. First, we confirmed dose-dependent induction of DNA damage using a known genotoxicant, methyl methanesulfonate (MMS), in human cell lines derived from breast (Mean % Tail DNA ± SEM, Control: 12.5±2.8; 20 µg/ml MMS Treatment (3h): 53.9±1.9, p≤0.01), cervix (4.7±1.3; 51.9±2.4, p≤0.006), liver (8.0±4.1; 60.6±0.8, p≤0.004), and spleen (3.2±0.7; 50.1±0.7, p≤0.001). Next, we defined background levels of global (5-mC) methylation in these cell lines (5-mC % ± SEM for breast: 1.8±0.56%, cervix: 2.2±0.31%, spleen: 0.9±0.28%, liver: 1.5±0.47%), and characterized the dose-response kinetics to several agents of interest to the FDA, including chemotherapeutic, environmental and novel agents. We then demonstrated proof-of-principle for our assay by detecting hypermethylation after 20 µM hydroxyurea treatment (Difference in % Tail DNA with McrBC vs. buffer ± SEM: 30.1±3.1, p≤0.002), and hypomethylation with 0.1 mM 5-Azacytidine treatment (-6.3±0.9, p≤0.03). To date, these results demonstrate high sensitivity of the modified Comet assay for detecting as little as a 14% reduction in global DNA methylation in single cells. The successful application of this novel technology will aid in the hazard identification and risk characterization of FDA-regulated products. Furthermore, this assay will have utility in investigating the potential epigenetic mode of action of agents in target organs, since the assay is amenable to cells in culture or cells from any tissue.

1397 mESC-Based *In Vitro* Differentiation Models for Analyzing the Impact of Genotoxic Agents on Stress Response and Functionality of Vascular Cell Types

G. Fritz and T. Hennicke. *Toxicology, Heinrich Heine University, Duesseldorf, Germany*. Sponsor: R. Landsiedel.

Due to their barrier function, vascular endothelial cells are exposed to the highest concentrations of systemically present noxae. Correspondingly, it is feasible that endothelial damage defence mechanisms and regenerative processes essentially support vascular functionality. Here, we established a mouse embryonic stem cell (mESC) based differentiation model in order to comparatively investigate the DNA damage response (DDR) of vascular progenitors and differentiated cells types (i.e. endothelial-like (EC) and smooth muscle-like (SMC) cells) following treatment with ionizing radiation (IR) and the cardiotoxic anticancer drug doxorubicin (Doxo). Using a direct differentiation protocol, we generated EC- and SMC-like cells from mESC. Extensive analyses were performed in order to ensure that the differentiated vascular cell types express cell type-specific functions and, hence can be considered as a relevant model system for *in vitro* toxicity studies. The data obtained show that differentiation of mESC into EC and SMC is accompanied by congruent and unique alterations in DNA repair and DDR capacities. For instance, EC and SMC shared downregulation of genes involved in the repair of DSBs as compared to mESC. By contrast, expression of BER genes was particularly low in SMC as compared to EC. Apparently, loss of pluripotency and gain of cell type specific functionality goes along with complex alterations in DNA repair and DDR capacities1. mESC, EC

and SMC largely differed from each other regarding alterations in gene expression following IR and Doxo treatment. Surprisingly, low dose of IR and Doxo did not impair differentiation of mESC into functionally competent EC and SMC. In summary, we suggest that the established mESC-based differentiation model is a useful tool for studying the impact of genotoxins on stress responses and functions of vascular cell types. We consider it as an appropriate *in vitro* model for basic research in genetic and developmental toxicology and, moreover, for *in vitro* toxicological drug assessments. 1Hennicke T, Nieweg, K, Brockmann N, Kassack MU, Gottmann K, Fritz G (2015) mESC-based *in vitro* differentiation models to study vascular response and functionality following genotoxic insults. Toxicological Sciences, 144, 138-150

1398 Comet Assay in Rat Nasal Tissue

K. Pant[2], S. W. Bruce[2], S. Springer[2], M. Klug-LaForce[2], P. Sareen[1,2], L. J. Rausch[1] and R. Kulkarni[2]. [1]*AkzoNoble, Inc, Chicago, IL and* [2]*BioReliance by SAFC, Rockville, MD.* Sponsor: K. Pant.

The *in vivo* alkaline comet assay is used for the detection of DNA strand breaks in cells or nuclei isolated from multiple tissues of animals, usually rodents that have been exposed to potentially genotoxic materials. One of the main advantages of the Comet Assay is that it allows use of any tissue of interest provided single cell population is prepared from that tissue. For inhalation studies, nasal tissue is important as it is the first site of contact. Due to the size of the nasal tissue, collection and preparation of single cell population requires skilled technicians and validated technique, and hence Comet assay in nasal tissues is less routinely performed. To satisfy the validation requirements as per the OECD Comet assay guideline 489 and in order to build historical database in nasal tissue, male Sprague Dawley rats were dosed with vehicle (saline) and the positive control ethyl methanesulfonate (EMS) at 200 mg/kg using oral gavage. Five independent experiments with two groups of five animals in each were conducted. Nasal tissues were collected and processed to prepare single cell suspensions as per the Test Guideline. The comet assay results from these five experiments demonstrated that oral dosing of EMS resulted in significant DNA damage in the nasal tissue (% tail DNA range from 9.63 to 20.55) and the response is significantly higher than the vehicle control (% tail DNA range from 0.18 to 0.39). The work presented in this poster is a proof of concept experiment to validate nasal tissue extraction, cell suspension preparation in detail and to demonstrate that Comet assay can detect the DNA strand breaks in nasal tissues. In summary, this technique can be used to prepare single cell population to perform Comet assay on nasal tissues for inhalation studies.

1399 Improvements to *In Silico* Mutagenicity Predictivity of Aromatic Amines with Access to Proprietary Data

S. J. Canipa[2], D. S. Macmillan[2], R. V. Williams[2], P. Leavitt[1], M. Mack[1] and L. Custer[1]. [1]*Bristol-Myers Squibb, New Brunswick, NJ and* [2]*Lhasa Limited, Leeds, United Kingdom.* Sponsor: C. Barber.

Access to proprietary data is important for robust *in silico* predictive models, as toxicological models built predominately on public data sources tend to lack comprehensive coverage in proprietary pharmaceutical chemical space, including reactive synthetic process intermediates and impurities. Mutagenicity alerts in Derek Nexus are currently built using approximately 75% public data and 25% proprietary data; however, there are still gaps in the chemical space covered. By using wide-ranging sources of proprietary data, it is possible to fill these gaps and improve predictivity. As part of a confidential data sharing project with one of our members, Bristol-Myers Squibb Company (BMS), Lhasa Limited had on-site access to an Ames test data set of 1261 compounds. The data was anonymised and the compounds were analysed by clustering (a structural fingerprinting technique) and by-eye to identify unexplored chemical space. Following data analysis, a number of new alerts and alert modifications were discovered. Aromatic amines are an important class of compounds commonly found to have mutagenic activity, and from this data sharing, three new alerts have been identified; tricyclic aryl amines, triaryl aromatic amines and heterocyclic amines. Implementing these alerts to the existing knowledge base in Derek Nexus significantly improved our performance against the BMS data set. The sensitivity increased from 25% to 76% with only a slight drop in specificity (from 88% to 85%). Furthermore, we observed enhanced performance against our own in-house mutagenicity data set. These alerts, and other alert modifications discovered during the on-site data sharing project, will be released in an upcoming version of Derek Nexus and further data sharing initiatives are in progress.

1400 A Targeted Approach to Improving the Applicability Domain of *Salmonella* Mutagenicity (Q)SAR Models

M. T. Kim, J. W. Yoo, B. Minnier, N. Kruhlak and L. Stavitskaya. *CDER/DARS, US FDA, Silver Spring, MD.*

The International Conference on Harmonisation M7 guideline describes the use of (quantitative) structure-activity relationship models to assess the mutagenic potential of drug substance impurities in new and generic drug products. In order to maintain optimal predictive performance and utility, models need to be continually updated with new data to ensure coverage for new drug substances entering the regulatory pipeline. Additionally, limitations in predictive performance caused by inadequate structural representation in selected areas of chemical space should be addressed. A targeted approach to enhancing a training set ensures that the existing performance of a model is maintained for well-represented areas of chemical space, while areas with few or no structural examples are improved upon. In this study, the FDA/CDER *Salmonella* mutagenicity model (n=3,979) was assessed using MACCS (166-bit) 2D fingerprints to determine its representation of chemical space. The Tanimoto coefficient (Tc) for all pairs of compounds within the training set was calculated and compared to identify compounds and sub-structural features that were poorly represented (i.e., less than 3 neighbors or analogs with Tc < 0.5) or outside of the applicability domain. Using this method, 30 compounds were identified as well as 79 sub-structural features that were poorly represented in the training set. The training set was then enhanced for prediction of *Salmonella* mutagenicity by rationally selecting 349 new compounds compiled from Drug Bank and newly published corporate data. New sub-structural features included bi- and tri- phenyl substituted alkanes, cyanamide derivatives, di- and tri- substituted haloalkanes, dioxanes, hindered epoxides, hydrazines, hydrazones, isoxazole, and N-(hydroxymethyl)acetamide derivatives. A hierarchical clustering analysis of the rationally selected chemicals showed representation of an additional 69 structural clusters over which the new model can make a prediction, thus expanding the overall applicability domain.

1401 Deficiencies in Mitochondrial Fission and Fusion Sensitize the Nematode *Caenorhabditis elegans* to Cisplatin-Induced Larval Growth Delays and Alterations in Mitochondrial DNA Copy Number

L. L. Smith[2] and J. N. Meyer[2]. [1]*Nicholas School of the Environment, Duke University, Durham, NC and* [2]*Pharmacology/ITEHP, Duke University, Durham, NC.*

Cisplatin, or cis-diamminedichloroplatinum, is a common anticancer drug treatment that has toxicological side effects in humans. There is evidence that some of these side effects result from mitochondrial toxicity, but the extent to which this drug affects mitochondrial health, an important factor in many increasingly prevalent diseases, is not completely understood. In order to get a better understanding of the effects the drug may have on a patient with an established mitochondrial disorder, the animal model *Caenorhabditis elegans* was tested for developmental delays and DNA damage after treatment with cisplatin. These studies have focused on strains with mutations in the mitochondrial fission and fusion genes drp-1 and fzo-1, which are necessary to maintain mitochondrial homeostasis, and the wild-type N2 Bristol strain. Synchronized populations of larval stage 1 nematodes were incubated for 48 and 72 hours at 20°C in cisplatin concentrations of 0, 5, 10, or 20uM. After 48 hours, nematodes were picked and frozen for further analysis of DNA damage and copy number. After 72 hours, nematode growth was measured and recorded. In comparison to the wild-type strain, the drp-1 and fzo-1 strains showed significantly greater cisplatin dose-dependent delays in development, as evidenced by size measurements (Two-way ANOVA, $p < 0.0001$). Nuclear genome copy number (Two-way ANOVA, $p < 0.0001$) and mitochondrial genome copy number (Two-way ANOVA, $p < 0.0001$) were also more impacted by cisplatin in the mutant strains than in wild-type; at the highest dose, the fzo-1 strain had significantly fewer mitochondrial copies per cell (Student's t-test, $p = 0.0158$). Above-background levels of DNA damage were not detected in either genome at these doses. Future work will include several unexplored mitochondrial mutant *C. elegans* strains, as well as additional mitochondrial parameters as the study progresses.

1402 Correlation of the *Pig-a* Assay with Regulatory Genetic Toxicology Endpoints

J. Bhalli, R. E. Neft, C. Tebbe, K. McNeil, M. Chan, J. Vanosdol, V. Sharma, E. Shaw and R. Fiedler. *Covance, Greenfield, IN.* Sponsor: G. Weinbauer.

Caffeic acid is present in a variety of fruits, vegetables and seasonings. It is considered to be a possible human carcinogen (Group 2B, IARC). It is negative in the Ames assay, positive for chromosome aberrations in CHO cells and negative for MN in mouse bone marrow. The Pig-a assay could be a useful follow-up for compounds that produce an equivocal or weak positive response in the Ames and/or *in vitro* mammalian cell cytogenetic assays. The objective of this study was to evaluate gene mutation in rats exposed to caffeic acid to determine the sensitivity of the Pig-a assay compared to the *in vivo* Comet and micronucleus (MN) assays that are frequently used in a regulatory setting. For this study two sets of six rats per group were dosed with vehicle (0.5% hydroxypropyl methylcellulose), 500, 1000 or 2000 mg/kg/day of caffeic acid for three consecutive days via oral gavage. One set of animals was used for the Pig-a and MN assays and one set was used for the Comet assay. Positive control animals (six per group) were dosed with 20 mg/kg/day of ENU for three days. Approximately 150 µl of blood was collected for the Pig-a (Days -1, 14 and 30) and MN (Day 4) assays. Three hours following the last dose, liver was collected and cells were isolated for the Comet assay. Analysis of MN by flow cytometry showed that the percent micronucleated polychromatic erythrocytes (%MNPCEs) were 0.08, 0.11, 0.14, and 0.20% for 0, 500, 1000 and 2000 mg/kg/day, respectively. The Pig-a assay showed no increase in the mutant frequencies of red blood cells (RBCCD59-) or reticulocytes (RETCD59-) in any tested dose group on Days 14 or 30. Positive control animals showed a robust increase in the RBCCD59- or RETCD59- frequencies. In conclusion, there were dose related increases in MN, but not in the Pig-a mutant phenotype erythrocytes of rats exposed to 500, 1000 or 2000 mg/kg/day caffeic acid. These results correlate with the lack of mutagenicity in the Ames assay and increases in chromosome aberrations observed *in vitro* and *in vivo* following exposure to caffeic acid.

1403 Reduced mtDNA Content Sensitizes *C. elegans* to Environmental Mitotoxicants

A. Luz and J. Meyer. *Nicholas School of the Environment, Duke University, Durham, NC.*

The integrity of mitochondrial DNA (mtDNA), which encodes 13 subunits of the electron transport chain, is intimately linked with energy production. This is highlighted by the fact that mtDNA mutations or depletion can cause disease in humans. For example, mutations in mitochondrial dNTP pool maintenance and mtDNA replication genes can cause mtDNA depletion syndrome (MDS), which is a heterogenous group of severe disorders that manifest early in life, and are characterized by a severe reduction in mtDNA content (>65%) and impaired energy production in effected tissues. Interestingly, mutations in the mitochondrial fusion gene (MFN2), and exposure to certain environmental toxicants or drugs, such as NRTIs, can also reduce mtDNA content, although typically to a lesser extent than MDS. As mtDNA is linked with energy production, and certain environmental toxicants can specifically target mitochondria, we hypothesized that reduced mtDNA content could sensitize an individual to mitochondrial toxicity. To address this question we reduced mtDNA content in the model organism, *Caenorhabditis elegans*, using low doses (1.0 µg/ml) of the DNA intercalating agent ethidium bromide (EtBr), and then exposed nematodes to known and/or suspected environmental mitotoxicants. Chronic exposure to EtBr reduced mtDNA content 40–55%, without effecting nuclear DNA content, nematode growth, ATP levels, basal respiration, proton leak, or ATP-linked respiration. These results suggest that this degree of mtDNA reduction has minimal effects on basal mitochondrial function. Next, nematodes with reduced mtDNA content will be exposed to the environmental mitotoxicants aflatoxin B1, arsenite, paraquat, rotenone, and ultraviolet C radiation (UVC), and ATP levels will be measured to test for exacerbation of mitochondrial dysfunction. Mitochondrial dysfunction will then be further characterized using the Seahorse XFe24 Analyzer to measure changes in the fundamental parameters of the mitochondrial respiratory chain. Preliminary results have demonstrated that reduced mtDNA content sensitizes nematodes to UVC exposure. Funded by NIEHS 1R01-ES017540-01A2.

1404 Relationship between Oxidatively-Induced DNA Damage and DNA Repair Enzyme APE1 in Cancer and Normal Cell Lines

L. D. Scanlan, B. C. Nelson, E. Coskun, A. Tona, M. Dizdaroglu and P. Jaruga. *National Institute of Standards and Technology, Gaithersburg, MD.*

Understanding the relationship between DNA damage and DNA repair is of utmost importance. Dysregulation of DNA repair can lead to disease such as cancer. Exploitation of DNA damage and repair mechanisms offers new avenues for cancer treatment, and it is therefore necessary to quantitatively measure DNA lesions and DNA repair enzymes in cancerous and normal cells to better understand the relationship between DNA damage and DNA repair. In this work, we used gas chromatography and tandem mass spectrometry (GC-MS/MS) with isotope dilution to measure the level of six oxidatively-induced DNA lesions (5-hydroxy-5-methylhydantoin, 5-hydroxy-cytosine, thymine glycol, 4,6-diamino-5-formamidopyrimidine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 8-hydroxy-guanosine) in four human cells lines (HeLa, HepG2, MCF7 and MCF10a). We also used liquid chromatography and tandem mass spectrometry (LC-MS/MS) with isotope dilution to measure the levels of the DNA repair enzyme APE1 in total protein extracts from cell cultures matched to those used for DNA extraction. We used both GC-MS/MS and LC-MS/ MS to measure S-8,5′-cyclo-2′-deoxyadenosine (S-cdA). We found all seven lesions to be present in all four cells lines, although at difference levels. For example, MCF7 had significantly higher levels of FapyAde, FapyGua and ThyGly than MCF10a. The levels of S-cdA detected with GC-MS/MS and LC-MS/MS were in agreement, and showed similar levels to those found previously in a rodent model. APE1 was detected in total extracts from all four cells lines, and was highest in MCF7 cells, followed by HepG2 and MCF10a. Further work is currently underway to understand the relationships between the different lesions and DNA repair enzymes, and to measure APE1 in in cytoplasmic and nuclear protein extracts.

1405 POLD3 Mutation Compromise Somatic Hypermutation in Human Burkett's Lymphoma Cells

W. Wu, Z. Xiao, I. Ban and V. Poltoratsky. *St. John's University, Jamaica, NY.*

Somatic hypermutation (SHM) acts as a pivotal role for B cell to interact with various foreign pathogens. SHM diversifies B cells' immunoglobulin gene and increase the affinity of B cell antibodies to antigens. The molecular process of SHM is initiated with the conversion of cytosine to uracil by the enzyme named activation-induced cytidine deaminase (AID). The mutated urail:guanine mismatch are then removed by a base excision repair pathway to the uracil base and formed an apurinic/apyrimidinic (AP) site. Following incision of the AP site by AP endonuclease, error-prone DNA polymerases are then recruited to gap and cause single point mutation. POLD3 is the one of the subunit of DNA polymerise δ, which has been confirmed not essential in yeast strains' viability. However, previous studies demonstrated that POLD3 is crucial for DNA damage repair in yeast. In mammalian cells, it is also shown that POLD3 subunit is recruited to sites of DNA damage in 293T cell line. On the basis of previous studies, we hypothesized that POLD3 is involved in the gap filling in SHM. In order to test the hypothesis, we construct a POLD3 truncated model in human Raji cells by the CRISPR technology and investigate the function of POLD3 in Raji cells' somatic hypermutation. Our results shown that POLD3 protein was significantly less expressed in the POLD3 gene truncated cell line (Raji 2C9 cells). Raji 2C9 cells also exhibited significant decrease of cells viability after expose to genotoxic agents, which is consistent with the results observed in yeast. However, the POLD3 mRNA level in Raji 2C9 cells are almost the same as that in Raji cells expressing the wild type POLD3. We further tested the effects of POLD3 on SHM in Raji cells by checking the mutagenesis at the immunoglobulin gene V-region. Our results indicate that SHM is significantly compromised in POLD3 truncated cells.

1406 Detection of DNA Double-Strand Breaks in the Liver of Mice Exposed to 1,2-Dichloropropane

R.-S. Wang, T. Toyooka, Y. Yanagiba and M. Suda. *Japan National Institute of Occupational Safety and Health, Kawasaki, Japan.*

1,2-Dichloropropane (1,2-DCP) is believed to be the causative substance of the biliary duct cancer which was reported at a high incidence rate among the employees in an off-set printing factory in Japan. However, it is not known whether and how 1,2-DCP induces any genetic damages in its carcinogenetic effect. So far we found it may induce damage on DNA as detected by comet assay in liver tissue, but this is not always

consistent in our experiments. In this study, we utilized histone H2AX phosphorylation, currently attracting attention as a new biomarker for DNA damage, and direct detection of DNA double-strand breaks (DSBs) by pulsed field gel electrophoresis (PFGE) to confirm the genotoxic effect of the solvent. Male C57BL/6J mice at 8 weeks old were exposed to 1,2-DCP at 0, 100, 200 and 400 ppm for 6 hr in the first day and then 3 hr in the second day. Mice were sacrificed 2 hr after the termination of inhalation exposure. Biochemical and histopathological evaluation of liver injury as well as DNA damage analyses were performed. 1,2-DCP exposure did not increase blood AST and ALT values. Ratios of liver to body weight were not affected either by the exposure. PFGE revealed that the level of DSBs formation was increased in a dose-dependent manner in 1,2-DCP exposed mice. This phenomenon was observed in both isolated liver cells and the whole liver tissue. Dose dependent generation of phosphorylated histone H2AX was also observed after 1,2-DCP exposure. These results suggested that 1,2-DCP exposure can induce DNA damages, including DSBs, one of the most relevant lesion for mutations and carcinogenesis, and this may launch the process for the occurrence of biliary duct cancer.

1407 Brominated Trihalomethane Toxicity in Human Urothelial Cell Lines

R. A. Pegram[2], J. A. Campbell[1], S. O. Simmons[2], T. M. Ross[2], D. M. DeMarini[2] and B. N. Chorley[2]. *[1]NCBA/SEE Program, Washington, DC and [2]US EPA, Research Triangle Park, NC.*

Epidemiology studies have consistently found that greater exposure to drinking water disinfection byproducts (DBPs) is associated with an increased risk for bladder cancer. In 2010, Cantor et al. (Environ. Health Perspect. 118: 1545) reported that this increased risk was dependent upon the GSTT1+ genotype and the implied expression of the xenobiotic metabolizing enzyme, glutathione S-transferase theta (GSTT1). We previously found that GSTT1 activates the prevalent DBPs, brominated trihalomethanes (BrTHMs), to mutagenic intermediates (Pegram et al., 1997, Toxicol. Appl. Pharm. 144: 183; DeMarini et al., 1997, Environ. Molec. Mutagen. 30: 440). In the present study, we determined both cytotoxicity and the induction of micronuclei by the BrTHM, dibromochloromethane (DBCM), in a human urothelial cell line (SV-HUC1) derived from normal human uroepithelium and in a novel transgenic line (SV-HUC-T1) that we engineered to stably overexpress human GSTT1. The SV-HUC1 line was genotyped for GSTT1 and found to be GSTT1+/+. In the SV-HUC-T1 culture used in these experiments, GSTT1 activity, 1,2-epoxy-3-(4-nitrophenoxy)propane conjugation by glutathione, was 77% higher than in the SV-HUC1 parent line. Cell viabilities in the SV-HUC1 and SV-HUC-T1 lines, respectively, were 88% and 80% after 24 hr exposure to DBCM at 4 mM and 86% and 74% at 6 mM. Compared to vehicle (DMSO)-treated controls, DBCM at nominal concentrations of 3 mM and 6 mM induced 2.1- and 5.3-fold increases in micronuclei per 1000 binucleated cells in the SV-HUC1 line and 3.1- and 7.7-fold increases in the SV-HUC-T1 line. Actual media concentrations of DBCM were estimated to be ~80% lower than nominal concentrations due to volatilization to headspace and adsorption to plastic in the micronuclei experiments. This is the first report of BrTHM-induced genotoxicity in human urothelial cells, and the first demonstration of GSTT1-dependent BrTHM genotoxicity in eukaryotic cells. [This abstract does not reflect EPA policy.]

1408 BDE-100 Has Genotoxic But No Mutagenic Potential

V. Bruno[2], L. C. Pereira[2], M. F. Franco-Bernardes[2], G. Meireles[2], M. J. Tasso[2], D. P. de Oliveira[2] and D. J. Dorta[2]. *[1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil and [2]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil.*

Flame retardants have been used on industrial scale in order to enhance the resistance of the fire and the high temperatures of various products. Due to their low cost, polybrominated diphenyl ethers (PBDEs) are the most common. However, it was observed that human exposure to BDE-100 might cause deleterious health effects. Endocrine disruption and liver toxicity were the first toxic effects described of these compounds, but their mechanism of action isn't well described. HepG2 and *Salmonella* strains were used in an attempt to elucidate its effect on DNA and its genotoxic and mutagenic potential. Thus, this research addresses the genotoxic and mutagenic potential of PBDEs. HepG2 was maintained in "MEM" supplemented with 10% fetal bovine serum in 5% of CO2 at 37 °C and evaluated by different endpoints after exposure to BDE-100. First, the genotoxic potential of the compound was evaluated through the comet assay and micronuclei formation using HepG2 culture cell at concentration from 0,1 to 25 µM of BDE-100. Then, the *Salmonella*/microssome assay was performed in Salmonella strains TA98 and TA100 exposed at concentrations from 0.025 to 10 µg/plate. The effects of HepG2 cells and *Salmonella typhimurium* exposure to BDE-100 were evaluated. The comet assay revealed that BDE-100 exerted genotoxic effects at higher concentration, but induced no micronuclei formation in HepG2 cells. This BDE has no significant mutagenic effects on the *Salmonella typhimurium* strains TA98 and TA100. Taken together, the results of the genomic instability assays showed that BDE-100 may represent risks to the health of directly and indirectly exposed population as evidenced by the Comet assay data because the assessed BDE induce genotoxic effects in the HepG2 cell line. Supported by: FAPESP - Proc. 2012/13123-0
"The opinions, assumptions, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of FAPESP"

1409 Detection of DNA Cross-Links in Rat Liver and Kidney in the *In Vivo* Alkaline Comet Assay

C. Beevers, G. Pearce, L. Foster and R. Mortimer. *Covance Laboratories Ltd, Harrogate, United Kingdom.* Sponsor: J. Bhalli.

In 2014 OECD published a test guideline for the conduct of the *in vivo* alkaline comet assay in rodents (OECD TG 489). The alkaline comet assay detects DNA strand breaks, which can be indicative of many of the genotoxic events, however, significant modifications to the test guideline protocol are required if DNA crosslinks are suspected as these cannot be reliable detected under standard conditions. A possible modification requires co-treatment (either *in vivo* or *ex vivo*) with a reference article that is known to cause DNA strand breaks. If the substance under investigation does not produce crosslinks in the DNA, then treatment with the reference article will result in an increase in DNA strand breaks comparable with the control, however, if the test substance does produce crosslinks in the DNA, then these will counter the damage induced by the reference article, appearing as reduced DNA migration (ie fewer strand breaks) than the concurrent vehicle control. When testing any unknown substance, reliable positive controls that demonstrate sensitive detection of crosslinks are essential. In order to determine a suitable crosslinking positive control and appropriate modification protocols we dosed female Han Wistar rats with cisplatin by oral gavage at dose levels up to 25 mg/kg/day. 3 hours after administration, animals were euthanized and single cell suspensions of liver and kidney were prepared and layered onto agarose coated comet slides. The cells were exposed *in vitro* to varying concentrations of methyl methanesulfonate (MMS) for 2 hours at room temperature and then processed through the alkaline comet assay. In the absence of *ex vivo* treatments with MMS, cisplatin did not induce any significant changes in DNA migration in the liver or kidney. Following *ex vivo* treatment with MMS cisplatin treated animals showed a significant dose related decrease in DNA migration compared to control animals, thus confirming that the crosslinking activity of cisplatin could only be detected in the modified alkaline comet assay. Cisplatin was confirmed as a suitable positive control for detection of crosslinks in a modified comet assay.

1410 Immunomagnetic Enrichment of CD71-Positive Reticulocytes Enhances the Efficiency and Precision of Human Blood Micronucleated Reticulocyte Scoring via Flow Cytometry

S. Avlasevich[1], D. Torous[1], J. Bemis[1], Y. Chen[2] and S. Dertinger[1]. *[1]Litron Laboratories, Rochester, NY and [2]Radiation Oncology, University of Rochester Medical Center, Rochester, NY.*

The frequency of micronucleated reticulocytes (MN-RET) among peripheral blood reticulocytes (RET) is a well-established indicator of chromosomal damage. An automated scoring method has been described whereby methanol-fixed blood samples are incubated with anti-CD71-FITC and anti-CD61-PE to label reticulocytes and platelets respectively, and with propidium iodide in conjunction with RNase to serve as a DNA dye (commercially available as *In Vivo* MicroFlow®). The current studies evaluated whether immunomagnetic enrichment of CD71-positive RET prior to flow cytometric analysis would significantly enhance the efficiency and precision MN-RET scoring. CD-1 mice were exposed to a range of gamma radiation up to 6 Gy. The 48 hr post-irradiation blood samples were scored for MN-RET frequencies according to the standard MicroFlow method and also using a RET enrichment scheme based on a FITC selection cocktail from StemCell Technologies. As expected robust dose-related increases in %MN-RET were where observed, with concomitant reduction to %RET. While flow cytometric analyses required shorter amounts of time for RET-enriched samples (avg 9 sec versus 429 sec), the mouse blood MN-RET frequencies were quantitatively similar between the methods. An analogous enrichment scheme using Miltenyi's MACS platform (with anti-FITC Microbeads) exhibited greater advantages for

human samples, especially those with low starting RET frequencies. While %MN-RET were correlated for blood samples from 49 different human subjects (R^2 = 0.84), both data acquisition time and reproducibility of technical replicates was improved with immunomagnetic separation. On average the RET-enrichment scheme provided slightly lower %MN-RET values, suggesting these values may be more accurate due to reductions to non-target cells and/or subcellular events.

1411 Qualification of Upper Respiratory Tract Tissues for Inhalation Exposure of Transgenic Big Blue® Mice

R. R. Young and H. Dinesdurage. *Toxicology, BioReliance by SAFC, Rockville, MD*. Sponsor: K. Pant.

A major advantage of the Big Blue® (BB) Transgenic Rodent (TGR) mutation assay is the ability to investigate tissue specific mutagenic mode of action (MOA) following positive genotoxicity and/or carcinogenicity results in mice and rats. As inhalation exposure is often a significant route of human exposure to chemicals, *in vivo* genotoxicity and 2 year carcinogenicity studies are frequently dosed via inhalation. Use of inhalation exposure in BB TGR assays required qualification of upper respiratory tract tissues (nasal cavity epithelium and lung) and demonstration that induced mutants can be observed in the URT tissues. This work describes methods to collect nasal cavity and lung tissue and optimize DNA extraction methods. We then report cII mutant frequency (MF) in vehicle control and positive control animals (40 mg ENU/kg/day PO days 1 to 3; necropsy day 31). Due to the small quantity and nature of nasal cavity tissues, modified tissue collection and DNA extraction methods were developed. Nasal tissue was collected from left and right nasal cavity by removing respiratory and olfactory epithelium with associated turbinates and septum. Modified DNA isolation methods were developed for nasal epithelium that included pulverization in a liquid nitrogen-cooled mortar and pestle, homogenization, nuclei pelleting, digestion and phenol chloroform extraction. Mutant analysis was possible with tissue from one side of the nasal cavity leaving the remaining side available for other analyses such as TK, gene expression or other genotoxicity assays such as DNA adduct or Comet. Lung was extracted using standard homogenization, digestion and dialysis methods using RecoverEase kits from Agilent. Background MF obtained from five studies were consistent with other somatic tissue MF. Statistically significant increases in cII MF were observed in lung (57.4 ± 15.0 x 10-6 in controls vs 154 ± 24.0 x 10-6 in ENU treatment groups) and nasal samples (54.4 ± 10.8 x 10-6 vs 416 ± 54.0 x 10-6). In comparison, similar values were obtained in liver (42.9 ± 19.0 x 10-6 vs 179 ± 44.4 x 10-6), glandular stomach 42.8 ± 15.0 x 10-6 vs 796 ± 205 x 10-6) and bone marrow (40.6 ± 16.2 x 10-6 vs 587 ± 225 x 10-6). These results extend the tissue range of the BB TGR mouse mutation assay to upper respiratory tract tissues thereby demonstrating robustness of the assay and the assay's suitable for use with inhalation exposure.

1412 Acute Exposure to DEHP and Its Metabolite MEHP Cause Genotoxicity and Mutagenesis in Mammalian Chinese Hamster Ovary Cells

Y.-J. Chang, P.-Y. Lin, C.-Y. Tseng and M.-W. Chao. *Biomedical Engineering, Chung Yuan Christian University, Taoyuan, Taiwan; Bioscience Technology, Chung Yuan Christian University, Taoyuan, Taiwan*. Sponsor: M.-W. Chao.

DEHP, Di-(2-ethylhexyl) phthalate, the most common phthalate plasticizer used in the production of polyvinyl chloride. *In vivo* studies have shown that DEHP can be metabolized in the intestine and converted to mono-ethylhexyl phthalate (MEHP). In addition, epidemiology studies have shown an association with elevated induction of rat hepatic cancer and developmental toxicity in the reproduction system in response to MEHP exposure. However, the mechanism of carcinogenicity induced by MEHP treatment remains unclear. Thus, we used four Chinese hamster ovary cells including NER-proficient AA8, NER-deficient UV5, BER-deficient EM9, and gpt transgenic AS52 as model. These cells were exposed acutely to MEHP for 2 hours and cultured for additional 24 h prior to the experiments. Results show that DEHP and MEHP caused dose-dependent decreases in cell survival, but with very different potencies: MEHP has the high potency; but DEHP is lower. Exposure to these compounds induced dose-dependent production of ROS, as determined by Cm-H2DCFDA fluorescence. Comet assay shows the DNA damages in the trend as same as cytotoxicity. It was noted however that simultaneous treatment of ROS scavenger N-acetylcysteine shows the protection against MEHP induced DNA damages. The cell cycle arrest in DNA damaged cells and mismatch DNA repair dysfunction after MEHP treatment. In 6-thioguanine selection assay, our data indicate that exposure to MEHP would cause a dose-dependent increase in mutant fraction to four fold of the untreated control. Then the mutant clones were collected and analyzed with mRNA array assay. It indicated that several genes that are able to stimulate metastasis in cancer cell were up-regulated in this mutant CHO cell. In summary, DEHP and MEHP are not only cytotoxic, but also mutagenic to the mammalian CHO cell.

1413 Coexposures to Inorganic Arsenic and MMS Initiates Synergistic Responses Associated with Cellular Carcinogenesis

A. Y. Efremenko, P. Balbuena, H. J. Clewell and R. A. Clewell. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Inorganic arsenic (iAs), a known human carcinogen, is thought to act primarily as a co-mutagen - exacerbating effects of other genotoxic chemicals. Methyl methanesulfonate (MMS) is an alkylating agent that induces DNA strand breaks. The purpose of our study was to determine whether combined exposure to iAs and a genotoxic compound (MMS) has a synergistic effect on DNA damage response and to identify the mechanism by which iAs affects MMS genotoxicity. We assessed mutagenicity (micronucleus; MN), DNA damage (Comet assay, pH2AX), and markers of cellular response, including Bcl2 (anti-apoptotic protein), NQO1 (prevents formation of radical species), p53 (modulates responses to DNA damage). Low (3 nM) and high (300 nM) doses of iAs were combined with 1, 3, 10, 30, and 100 µM MMS for 24hr in HT-1080 cells. Validation of this cell model was achieved by comparing responses in HT-1080 and primary human uroepithelial cells (target for arsenic-induced bladder cancer) to several concentrations of arsenates. Both *in vitro* systems showed similar p53 and NQO1 responses to arsenates when compared for system validation. iAs/MMS mixtures significantly increased protein expression for Bcl2 and NQO1, (>2-fold) compared to MMS alone. In contrast, iAs slightly reduced MMS induction of p53. iAs had negligible effect on MN induction, but had a greater than additive effect on DNA damage (Comet, pH2AX). Gene array studies with MMS alone vs. iAs/MMS mixtures showed increased DSB repair (NHEJ) in the presence of iAs. Transcriptomics also confirmed repression of apoptosis and repression of p53-mediated signaling by upregulation of MDM2 via NFAT1. Together these protein, gene and genotoxicity assays support a co-mutagenic mode of action for iAs through inhibition of apoptosis and p53-mediated single strand break repair.

1414 Generation of a Double-Gene Knockout Mammalian Cell Line Using CRISPR Technology

D. Burris and V. Poltoratsky. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Organisms are exposed to toxic environment pollutants that introduce lesions in the DNA and the accumulation of lesions elevates the frequency of mutations in DNA. The mutagenic effects of endogenous and exogenous mutagens are altered by the defense mechanisms of the cell, including DNA repair. Pol ß null mouse fibroblasts, deficient in base excision repair (BER), have an elevated frequency of mutations, compared with isogenic wild-type cells, after treatment with the model alkylating agent, methyl methanesulfonate (MMS). Despite the fact that MMS is only weak mutagen in mouse cells, in the absence of pol ß in mouse fibroblast, MMS-induced mutagenesis is significant and involves base substitutions of all types. Although the mechanism of this effect is unknown, it is possible that the pol ß deficiency makes way for other DNA polymerases that bypass the alkylated lesions in an error-prone manner. We previously demonstrated that, error- prone, DNA polymerase REV1 is required for MMS-induced mutagenesis in pol ß null mouse fibroblast cells. It is interesting that the spectrum of mutations introduced by REV1 could not be explained by nucleotidyl transferase activity of this polymerase. Here we described the method to generate polβ-/-, REV1-/- double knockout mouse embryonic fibroblasts to determine whether abrogation of REV1 polymerase ameliorates BER activity in cells. We used the CRISPR approach targeted to REV1 to achieve a stable reduction in the steady-state level of the protein in pol β null cells and examined the frequency of MMS-induced mutations. Expression level of REV1 protein in mouse embryonic fibroblast pol β null cells was negligible compare to wild type cells. Down regulation of REV1 expression reduced the MMS-induced mutagenesis in pol β null cells. We plan to create a series of cell lines expressing different REV1 constructs, including, wild type REV1 (REV1WT), nucleotidyl transferase deficient (REV1DE/AA) mutant, and REV1-1, and test the frequency of mutation in these cells.

1415 Flow Cytometry for *In Vitro* Assessment of Genotoxic Substances

G. Marceau, F. Merah, S. Lavallée, F. Leroux and A. Nelson. *ITR Laboratories Canada, Baie d'Urfe, QC, Canada.* Sponsor: X.-X. Li.

Flow cytometric analysis offers new possibilities in genetic toxicology by providing an easy way to increase both the number of samples and the number of cells per sample, therefore increasing the statistical power of the results. Here we detail the implementation of the micronucleus assay by flow cytometry for the purpose of *in vitro* genotoxicity assessment. CHO-K1 cells were exposed to either vehicle or known genotoxins (Cyclophosphamide, CPA; Colchicine, CLC; and Mitomycin C, MMC) in two modes of exposure, short (approx. 4 hours with additional 24 hours in normal media) and extended (approx. 26 hours). Dead cells were tagged with Ethidium Monoazide (EMA) and then lysed and labeled with a DNA dye. DNA fluorescence intensity was acquired on FacsCanto II flow cytometer for the quantitation of haploid/diploid nuclei, hypodiploid nuclei and micronuclei. The relative population doubling of each sample was calculated with the help of fluorescent beads. In short exposure controls the relative amount of hypodiploid nuclei and micronuclei were 0.2% and 1.3% respectively. With CLC from 0.1 to 0.3 ng/mL, the relative amounts of hypodiploid nuclei were from 0.2% to 3.0% and micronuclei from 1.5% to 6.1%. In short exposure of controls with metabolic activation, the relative amount of hypodiploid nuclei and micronuclei were 0.4% and 2.9% respectively. With CPA from 1 to 3 ng/mL, the relative amounts of hypodiploid nuclei were from 0.6% to 0.7% and micronuclei from 6.0% to 13.5%. In extended exposure of controls, the relative amount of hypodiploid nuclei and micronuclei were 0.1% and 1.2% respectively. With MMC from 0.05 to 0.25 ng/mL, the relative amounts of hypodiploid nuclei were from 0.4% to 0.5% and micronuclei from 5.2% to 11.6%. Exposure with CPA and MMC induced measurable dose-dependent increases in micronuclei in the short and extended protocols. Exposure with CLC induced measurable dose-dependent increases in both hypodiploid and micronuclei. The analysis of *in vitro* nuclei by flow cytometry provide an alternative to microscopic slides reading for detecting both aneugens and clastogens, with capacity for high throughput.

1416 Investigation of Cytotoxicity and Genotoxicity of Caffeic Acid, a Dietary Bioactive Compound, in Association with 5-Azacytidine in Jurkat Cells

L. C. Hernandes[2], C. d. S. Machado[1], A. R. Machado[2], D. L. Ribeiro[1], R. V. Burim[2], M. d. L. Bianchi[2] and L. M. Antunes[2]. [1]*School of Medicine of Ribeirão Preto, USP, Ribeirao Preto, Brazil and* [2]*School of Pharmaceutical Sciences of Ribeirão Preto - University of São Paulo, Ribeirao Preto, Brazil.* Sponsor: D. Palma.

The influence of dietary bioactive compounds in epigenetic modulation has been evidenced in recent years. Caffeic Acid (CA) is a polyphenol widely distributed in human diet, being found in many foods, especially coffee. Besides its antioxidant activity, some studies have indicated that CA inhibits DNA methylation, therefore it is interesting to investigate the effects of this dietary bioactive compound in association with the demethylating agent 5-azacytidine (AZA), which acts by altering the pattern of DNA methylation in the treatment of myelodysplastic syndromes and leukemias. The aim of this study was to evaluate the cytotoxicity and genotoxicity of CA associated or not with AZA in leukemic human cells Jurkat. The cytotoxicity was evaluated by the MTT and Neutral Red (NR) Assays, and the DNA damage was determined by the comet assay. For MTT and NR assays, the cells were treated with CA (1-250 µM) and AZA (0.5-50 µM) or their association for 24 hours. For comet assay, cells were treated with CA (1; 10; 100 µM), AZA (5 µM), or their association for 4 hours. The viability of Jurkat cells exposed to CA was greater than 90% in both assays, while the treatments with AZA at concentrations above of 7.5 µM decreased significantly the cell viability compared to negative control group. No significant increase of % DNA in tail was observed in treatments with CA, AZA or their association. In conclusion, CA alone or associated with AZA did not induce cytotoxicity or genotoxicity in Jurkat cells. Follow studies will be carried out to establish the effects of CA and AZA in the DNA methylation pattern. Financial support: FAPESP(process: 2013/13733-6).

1417 Extreme Expressions of DNA Repair Proteins APE1 and MTH1 in Human Breast Cancer as Measured by Liquid Chromatography and Isotope Dilution Tandem Mass Spectrometry

E. Coskun[1,3], P. Jaruga[3], A.-S. Jemth[4], O. Loseva[4], L. D. Scanlan[3], A. Tona[3], M. S. Lowenthal[3], P. Reddy[3], T. Helleday[4] and M. Dizdaroglu[3]. [1]*Gazi University, Ankara, Turkey;* [2]*Biomolecular Measurement Division, MML, National Institute of Standards and Technology, Gaithersburg, MD;* [3]*Biosystems and Biomaterials Division, MML, National Institute of Standards and Technology, Gaithersburg, MD and* [4]*Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.*

Accurate measurement of DNA repair proteins in cancer tissues is becoming more important due to the individual and origin based expression differences in cancer patients as well as the novel approach of using the repair enzyme inhibitors in cancer treatment. However, available methods for measuring protein levels such as gel-based systems are indirect and not quantitative. Here, we applied an approach using liquid chromatography-tandem mass spectrometry with isotope dilution to accurately measure protein levels in normal and cancerous human breast tissues. We observed extreme expression of APE1 and MTH1 in malignant tumors when compared to disease-free breast tissues, suggesting that breast cancer cells may require these proteins for survival. The novel approach described herein is potentially applicable to the accurate measurement of DNA repair proteins' expression levels in cancerous vs. normal tissues in patients. This approach may help use APE1 and MTH1 as reliable biomarkers for the development of novel cancer treatment strategies and inhibitors of these proteins as anticancer drugs leading to personalized therapies.

1418 Flow Cytometry and Microscopy Method Comparison for *In Vivo* Micronucleus Studies in Rat Bone Marrow and Peripheral Blood

F. Merah, M. Charlebois, S. Thebault, J. Godin-Ethier, G. Marceau and A. Nelson. *ITR Laboratories, Montreal, QC, Canada.* Sponsor: X.-X. Li.

With the adoption of the ICH S2(R1) the limitations associated with using rat peripheral blood have been overcome by using flow cytometry. Among the advantages of this method are: low blood volume requirements, a high number of cells scored, scoring of very young immature erythrocytes, and the ability to sample without sacrificing the animals. Here we compared the relative strengths and limitations of microscopic and flowcytometric methods for micronuclei scoring in rat bone marrow and peripheral blood. Five animal replicates were treated independently at 24 hours intervals. In each replicate one group was treated with Cyclophosphamide at 20 mg/kg via intraperitoneal (i.p.) injection and a second group was either treated i.p. with 0.9% sterile saline solution or with purified water via oral gavage as vehicle controls. Bone marrow was collected 24 hours post-treatment and evaluated by fluorescence microscopy. Peripheral blood was collected 48 hours post-treatment and evaluated by fluorescence microscopy using supravital staining and by flow cytometry. At least 2,000 erythrocytes were evaluated by manual method and over 10,000 by flow cytometry. Overall the results were as expected with all three methods. There were low micronuclei frequencies with the vehicle controls and a significant increase in micronuclei frequencies in cyclophosphamide-treated groups. However, the results percentages obtained with the bone marrow scored manually and peripheral blood scored automatically were closer: (0.13, 1.99), (0.13, 1.57), with vehicle and cyclophosphamide, respectively. The results obtained with peripheral blood stained supra-vitally were less evident (0.08, 0.67). When using the supra-vital method it was difficult to select enough young immature erythrocytes for scoring. The flow cytometry is clearly superior for analysis of peripheral blood while the standard fluorescent microscopy remain reliable method for analysing bone marrow preparation.

1419 Profiling Genetic Susceptibility to Food Carcinogens Using Budding Yeast

M. Fasullo, J. Freedland and N. St. John. *SUNY Polytechnic Institute, Albany, NY.* Sponsor: M. Fasullo.

The human response to environmental carcinogens is highly variable. Environment, lifestyle, and genetics are factors that influence bioactivation. Genetic factors include polymorphic P450 and DNA repair genes; however, epidemiological studies may lack significance due to inadequate patient numbers. We used budding yeast as a model organism to determine genetic susceptibility to food-associated carcinogens, includ-

ing aflatoxins (AFB1) and heterocyclic aromatic amines (HAAs). Budding yeast does not contain P450s that activate these compounds, so we introduced expression vectors that contain specific human P450 and NAT2 genes. In yeast, either CYP1A2 or CYP1A1 activates AFB1, while both CYP1A2 and NAT2 are required for activation of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ). To measure genotoxic effects, we measured recombination and mutation frequencies, Rad51 foci, growth inhibition and DNA adducts, as in a previous publication concerning CYP1A2 polymorphisms. Here, we analyzed two CYP1A1 polymorphisms, T461N and I462V, correlated with breast and lung cancer. Although some studies have suggested that these polymorphisms confer reduced activity, both CYP1A1 polymorphisms are highly efficient at activating the AFB1 and benzo[a]pyrene dihydrodiol (BaP-DHD). To determine resistance genes, we used a high throughput approach for screening the yeast deletion library expressing either CYP1A2 or CYP1A2 and NAT2. Screens for aflatoxin resistance identified checkpoint and RNA metabolism genes that are mutated in cancers. We are now performing screens to identify genes involved in resistance to IQ. Preliminary data identified both recombinational repair and DNA damage tolerance genes. Further high throughput analysis will be performed using other food carcinogens, including 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). Grant Support: National Institutes of Health, 1R15ES023685-01

PS 1420 Evaluation of Genotoxic Effects Induced by Tio2 and ZnO Nanoparticles

J. J. Kim. *Sungkyunkwan University, Seoul, Korea, Democratic People's Republic of.* Sponsor: D. Y. Shin.

There are many toxic researches in the different kinds of nanoparticles. However, it is hard to utilize results of studies due to the diversity of physico-chemical properties such as different nanoparticle sizes, shape and surface charges. The aim of our study is to make it uniform dispersion conditions followed by NANOGENOTOX dispersion protocols and evaluate the genotoxicity of TiO2 and ZnO nanoparticles. At first, the benchmark materials (SiO2) were used to establish nanoparticle dispersion conditions. Dynamic light scattering (DLS) measurement was used for the size distribution. The comet assays were performed to evaluate the genotoxic effects in the human normal bronchial epithelial BEAS-2B cells. Wst-1 as cytotoxicity test was conducted to find proper nanoparticle-exposed concentrations in genotoxic test. The dispersion condition was determinated at 25% amplitude for 16 min using SiO2 nanoparticles. The average size of TiO2 and ZnO in a 2.56mg/ml stock dispersion was 474.3 ± 44.96 nm and 208 ± 16.10 nm, at the same dispersion conditions. When exposed to TiO2 and ZnO in BEAS-2B cells, No cytotoxic effects of TiO2 is shown, except for ZnO in wst-1. In addition, DNA damage in genotoxicity test was found in a dose-dependent manner only at ZnO. Overall, ZnO has shown the genotoxic effects, not TiO2 on BEAS-2B cells.

PS 1421 *In Vitro* Genotoxic Assessment of PCB3 Metabolites

S. Flor and G. Ludewig. *OEH, University of Iowa, Iowa City, IA.*

Semivolatile lower chlorinated biphenyls (PCBs) have been identified in inner city air, as indoor air pollutants in schools and other buildings, and at many other sites. Inhalation exposure to these compounds is of great concern in light of new studies revealing that at least one such compound, 4-monochlorobiphenyl (PCB3), has tumor initiating and mutagenic activity *in vivo*. PCB3 is readily converted by xenobiotic-metabolizing enzymes to mono- and dihydroxy-metabolites and quinones. *In vitro* all tested metabolites produced micronuclei and PCB3 quinones (PCB3-pQ; 4-chloro-2`,5`-dioxobiphenyl; PCB3-oQ; 4-chloro-3`,4`-dioxobiphenyl) induced gene mutations. Only the catechol (PCB3-Cat; 4-chloro-3`,4`-dihydroxybiphenyl) induced sister chromatid exchanges and only the hydroquinone (PCB3-HQ; 4-chloro-2`,5`-dihydroxybiphenyl) polyploidization. Our hypothesis is that different mechanisms are involved in these unique genotoxic profiles, either reactive oxygen species or DNA adduction in the case of PCB3-pQ, and adduction to proteins responsible for mechanical functions of the cell in the case of PCB3-HQ. We investigated mitotic phase distribution, spindle aberration and centrosome amplification in cultured V79 Chinese hamster lung cells and measured ROS and GSH levels in normal and GSH reduced cells treated with PCB3-pQ or PCB-HQ. With PCB3-HQ, we observed a tetraploid intermediate and loss of individual chromosomes during following cell cycles, suggesting viable but genetically unstable tetraploid cells and aneuploid daughter cells. The formation of monoasters and multiple nuclei per cell suggest mechanical problems in PCB3-HQ exposed cells; intracellular ROS was increased and GSH levels were decreased. PCB3-pQ, its redox partner, caused strong cytotoxicity, inhibition of tubulin polymerization *in vitro*; a decrease in GSH levels and increase in ROS, suggest oxidative stress, possibly via depletion of the major antioxidant GSH as one mechanism for chromosome breaks and point mutations. Elucidation of how PCB3-HQ causes polyploidization requires further analysis. (Supported by NIEHS P42 ES013661)

PS 1422 Assessment of the Genotoxic and Mutagenic Potential of Acrylamide in Rats and Mice by Use of the MN and Pig-a assays

L. Recio[1], K. Shepard[1], J. Davis[1], M. Friedman[2], D. Marroni[2] and C. Hobbs[1]. *[1]ILS, Research Triangle Park, NC and [2]SNF, Andrézieux, France.*

Acrylamide is a rodent carcinogen that occurs at varying levels in a number of fried or baked foods. In F344 male rats and in B6C3F1 mice, 2-yr acrylamide exposure leads to an increase in rat thyroid follicular adenomas and adenocarcinomas and in mice and increase in lung and harderian gland tumors. In the present study, we evaluated the genotoxic and mutagenic potential of acrylamide in male F344/DuCrl rats and B6C3F1 mice using the micronucleus assay and the Pig-a mutation assay, respectively. F344/DuCrl rats were administered 0.0, 0.5, 1.5, 3.0, 6.0, or 12.0 mg/kg bw-day and B6C3F1 0.0, 1.5, 3.0, 6.0, 12.0 or 24 mg/kg bw-day acrylamide per day (n= 7 rats per dose group) in drinking water to cover and exceed the range of acrylamide exposures used in the cancer bioassays. Acrylamide exposure was for 30 -31 days. A positive control N-ethyl-N-nitrosourea (30 mg/kg) was administered on two separate sets of days (day 1-3) to meet the expression time for the Pig-a mutant phenotype, and days 28-29 appropriate for the MN assay. The frequency of immature (PCE) and mature (NCE) erythrocytes in peripheral blood containing micronuclei or Pig-a mutants was determined by flow cytometry. Dunnett's multiple comparison test was used to determine if a treatment group was significantly different ($p < 0.05$) from vehicle controls. The results of both the micronucleus and Pig-a assays were negative for male rats exposed to acrylamide at the concentrations tested. In mice the Pig-a assay was negative; the MN assay data was previously positive in this lab. We hypothesize that, in mice, this reflects the cytogenetic activity of acrylamide rather than its ability to induce gene mutations as measured by the pig-A assay. The relatively low levels of Cyp450 2E1 in rats and/or efficient detoxication of the parent compound and its reactive metabolite glycidamide by GSH-transferases results in a lack of micronuclei in rats.

PS 1423 Genotoxicity and Expression of Pro-Inflammatory Molecular Markers in Emissions from a 2010 Diesel Truck Equipped with Diesel Particulate Filter (DPF) and a Selective Catalytic Reduction (SCR) System

N. Kado[1,5], C. F. Vogel[5], P. Wong[1], K. Na[2], C. La[5], S. Yoon[1], J. Collins[1], A. Alvarado[1], J. Herner[1], W. Robertson[2], K. Sahay[2] and L. Smith[1]. *[1]California EPA, Sacramento, CA; [2]Air Resources Board, California EPA, El Monte, CA; [3]Air Resources Board, California EPA, Sacramento, CA; [4]Environmental Toxicology, University of California, Davis, CA and [5]Environmental Toxicology - Center for Health and the Environment, University of California, Davis, CA.*

As an approach to reduce toxic pollutants in vehicle emissions, a number of control strategies have been initiated. In the current study, we investigated emissions from a 2010 heavy duty (HD) diesel truck equipped with advanced emission controls and evaluated the genotoxicity and the expression of pro-inflammatory markers in the collected particulate matter (PM). The vehicle, a class 8 HD diesel truck with a 2010 Volvo D13 engine was equipped with a diesel particulate filter (DPF) and a selective catalytic reduction (SCR) system to control emissions. The vehicle was tested on a HD chassis dynamometer using two separate test cycles - a double urban dynamometer driving schedule (UDDS x2) and a 45-minute 50-mph steady-state cruise cycle. Commercially available ultra-low sulfur diesel (ULSD) fuel was used throughout. PM extracts were tested for genotoxicity using the Salmonella/microsome assay (microsuspension modification) and in the Comet assay using cultured human U937 cells. Expression of pro-inflammatory molecular markers (for example, IL-8, COX-2) were examined in the human U937-derived macrophage cells. Although emission levels were lower than reported for uncontrolled HD diesel vehicles, there were detectable genotoxic emissions for both the mutagenicity and for the Comet assays. Mutagenic emissions on a per mile and on a per mass basis (rev/ug) were highest in Salmonella tester strain TA98 (-S9) for both UDDS and Cruise test cycles. There were also detectable responses that induced expression of pro-inflammatory markers IL-8 and COX-2 in these samples.

1424 Identification and Characterization of *N7*-Guanine Adducts of 1-Bromopropane

M. R. Nepal, J. Ahn, K. S. Jeong, K. Noh, M. J. Kang and T. C. Jeong. *College of Pharmacy, Yeungnam University, Gyeongsan, Korea, Republic of.*

To minimize the use of chlorofluorocarbons, brominated compounds were introduced. Among them, 2-bromopropane (2-BP) was extensively used in several industrial processes, but the reproductive toxicity was reported in workers of an electronic company in Korea in 1995. The use of 2-BP was substituted with 1-bromopropane (1-BP). However, the toxicological behaviors of 1-BP were also reported to be developmental and neurological. A number of studies on bromopropanes have been conducted, but 1-BP adducts formed with DNA/RNA *in vivo* have not been studied yet. Thus, we focused to determine the amount of N7-guanine adducts in DNA and RNA of various tissues of rats. In addition, we tried to find out the DNA adducts in MCF-7 breast cancer cells. Two groups of rats were orally administered with 0.5 g/kg and 1 g/kg of 1-BP, respectively. One group of rats was administered with single dose, and the other group of rats was administered with repeated doses for 3 days. Rats euthanized after 6 hr of last administration. DNA and RNA were extracted using established protocols, and analyzed by LC-MS/MS. MCF-7 cells were treated with 1-BP at 50, 200 and 500 µg/ml for 12, 24, and 48 hr and DNA from cells were isolated and analyzed. N7- Guanine adducts could be determined in all tissues except in heart. Unlike DNA adducts, RNA adducts were determined in only kidney and spleen. Liver showed the maximum amount of DNA adducts in both studies, and the kidney exposed to 1 g/kg for 3 days showed the maximum amounts of RNA adducts. The highest level of DNA adducts was determined in MCF-7 cells treated at 500 µg/ml for 48 hr. The results indicate the possible role of adduct formation with nucleic acids in 1-BP induced toxicity.

1425 Formation and Repair of Exogenous Formaldehyde-Induced DNA-Protein Crosslinks

Y. Lai, X. Tian and J. Swenberg. *Environmental Sciences and Engineering, The University of North Carolina at Chapel Hill, Chapel Hill, NC.* Sponsor: J. Swenebrg.

Background: Formaldehyde (FA) is classified as an animal and human carcinogen. FA-induced DNA-protein crosslinks (DPCs) have long been thought to be a major form of DNA damage. Thus, DPC formation and repair are crucial for genomic integrity and consequently for understanding and preventing carcinogenesis. In the present study, we investigated the formation, persistence and repair of FA-induced DPCs in A549 cells. Methods: Formaldehyde-induced DPCs, specifically deoxyguanosine-Me-Cysteine (dG-Me-Cys) crosslinks, were measured by using highly sensitive nano-LC-MS/MS. After 3 hours of exposure with 150 µM [13CD2]-FA, cells were washed with PBS and incubated in fresh media for post exposure study. Results: After little change over the initial 2 hours post exposure, exogenous DPCs decreases rapidly from 19.0 to 11.0 crosslinks/108 dG at 2 and 4 hours post exposure, respectively. However, a much slower decrease in the number of exogenous DPCs was observed after 4 hours post exposure, and the number of DPCs was 9.0 crosslinks/108 dG at 8 hours post exposure. Actually, there was little change in the number of exogenous DPCs at 6 hours post exposure and beyond, showing long term persistence of some exogenous DPCs. In addition, we found DPC repair rate decreases with the addition of 2 µM MG-132, a widely used proteasome inhibitor. This evidence suggests that proteasome function contributes partially to the DNA damage response. In our previous animal study, we also demonstrated that exogenous FA-induced DPCs accumulated in rat noses over the 28-day exposure (2 ppm, 6h/day) and showed little change after either 24 or 168 hour (7 day) post exposure. Conclusions: The present study demonstrated that some FA-Induced DPCs show rapid repair rate with the involvement of proteasome activity. The long term persistence of some FA-induced DPCs highlights the need for better understanding of DPC repair.

1426 Integration of *In Vivo* Genotoxicity Assessment Including Multiple Endpoints and Tissues into a Repeat-Dose Toxicity Study

Z. Ji, L. K. Sosinski, N. Visconti, M. W. Koehler, N. S. Ball and M. J. LeBaron. *Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.*

An integrated toxicity testing strategy represents an important approach to generate comprehensive data and optimize animal use. The Pig-a assay, an *in vivo* mutagenicity test, is of interest due to its high integrability. The present study evaluated gene mutation via the Pig-a assay, clastogenicity/aneugenicity via the micronucleus test, primary DNA damage via the Comet assay, compared Pig-a and Big Blue® transgenic rodent mutation assay (TGR), and explored the Pig-a assay in sperm to detect germ cell mutagens. ENU (1, 10, or 20 mkd on study days 1-5 and 27-29), EMS (200 mkd on study days 27-29), or vinblastine (0.25 mkd on study days 27–29) increased MN-RET frequency and %tail DNA in liver, duodenum, kidney, and bone marrow in male F344 rats 3 hours after the last dose. The increase in gene mutation detected by the Pig-a assay was qualitatively consistent with the response in the TGR observed in bone marrow in animals treated with ENU (20 mkd on study days 1–3) or BaP (50 mkd on study days 1–28). A dose-related increase in frequency of CD59-deficient sperm was observed in rats treated with ENU (1, 10, or 20 mkd on study days 1–5) on study day 63 suggesting the Pig-a assay in sperm is a promising approach for detection of germ cell mutagens. Overall these results support the integrated genotoxicity testing strategy and suggest that the Pig-a assay is an animal saving alternative to the TGR assay for assessing *in vivo* mutagenicity.

1427 Down-Regulation of Poly (ADP-ribose) Polymerase 1 Leads to Change of Hydroquinone Cytotoxicity in TK6 Cells

Y. Sha. *Shenzhen Prevention and Treatment Center for Occupational Diseases, Shenzhen, China.* Sponsor: R. Lloyd.

Hydroquinone (HQ), one of the most important metabolites derived from benzene, is known to be associated with acute myelogenous leukemia risk; however, its carcinogenic mechanism remains unclear. In a previous study, we found that low-level of benzene exposure down-regulated the expression of poly(ADP-ribose)polymerase 1 (PARP1). Here, we employed RNA interference to knock down PARP1 expression in TK6 cells and explored the potential role of PARP1 in HQ-induced cytotoxicity. The results showed that stable PARP1-knockdown cells were successfully constructed and more than 80% inhibition of PARP1 expression was confirmed. We found that HQ treatment of TK6 cells decreased cell viability, increased cell apoptosis, and caspase3/7 activity. Knockdown of PARP1 in HQ-treated TK6 cells prevented caspase3 activation, and increased apoptosis than that in the wild-type TK6 cells, with fully functional PARP1. The results also showed that down-regulation of PARP1 led to a decrease in cell proliferation and an enhanced susceptibility to HQ-induced cytotoxicity with concentration less than 40 µM than that in normal TK6 cells. Moreover, PARP1-knockdown TK6 cells treated with HQ displayed an increased level of DNA double-strand breaks as measured by olive tail moment. No evidence was obtained of an effect of PARP1 depletion on H2AX phosphorylation induction. Under the experimental conditions, PARP1 has a role in HQ-induced DNA damage repair rather than in long-term chromatin modifications signaled by phosphorylated H2AX.

1428 Protective Effects of Parp Inhibitors at High Levels of Ros

I. Ban and V. Poltoratsky. *Pharmacy and Health Sciences, St. John's University, Jamaica, NY.*

In states of exposures to ROS, cells accumulate single stranded breaks in their genome. These breaks are rapidly marked by Poly ADP ribose polymerase (PARP), using NAD+ as a substrate. The activation of PARP and polymerization of poly (ADP-Ribose) represent one of the first steps in a coordinated series of events following single and double strand brake repair. Upon marking lesions with poly (ADP-Ribose), pathways such as base excision repair (BER) and non-homologous end-joining (NHEJ) repair the damaged DNA. At times of moderate oxidative stress several DNA lesions cause PARP activation and induce DNA repair pathways. In contrast at times of high oxidative stress PARP over activation, due to extensive DNA damage, depletes the cell NAD+ and consequently leads to necrotic cell death. In this study we tested the effect of newly synthesized PARP-inhibitors (compound 64, Compound 65 and Valiparib) cytotoxicity and genotoxicity during oxidative stress on lung cancer cell line. Exposure of A549 cells to nontoxic concentrations of PARP inhibitors decrease cytotoxicity of hydrogen peroxide compared to controls

(The controls include cells treated with DMSO not containing the drug). Experiments with flow cytometry show that the majority of cells die due to necrosis. Expression levels of DNA repair genes measured using RT-PCR profiling. This supports the hypothesis that cells deplete intercellular NAD+ and die of energy independed pathways

1429 Critical Evaluation of Air-Liquid Interface Cell Exposure Systems for *In Vitro* Assessment of Atmospheric Pollutants

M. A. Higuchi[3], A. Ledbetter[3], D. S. Morgan[2], S. D. McCullough[3], R. B. Devlin[3] and J. Zavala[1]. *[1]ORD, NHEERL, EPHD, ORISE Fellow, US EPA, Research Triangle Park, NC; [2]ORD, NHEERL, EPHD, Student contractor, US EPA, Research Triangle Park, NC and [3]ORD, NHEERL, EPHD, US EPA, Research Triangle Park, NC.*

We compared various *in vitro* exposure systems for their ability to expose cells to particles and gases. The systems tested use different mechanisms to deliver multi-pollutants to the cells: diffusion, sedimentation, thermophoresis (THP) and electrostatic precipitation (ESP). Various factors, such as flow rate, can adversely affect performance and reliability. We used fluorescent polystyrene spheres (50–1000-nm) as a surrogate for particulate matter to assess particle deposition. Deposition was determined by dissolving the spheres in ethyl acetate and measuring the fluorescence. Particles <100-nm rely on diffusion for deposition, whereas particles >1-µm rely on sedimentation. Applying external forces, such as THP or ESP, enhances deposition for all particle sizes. For example, with a 50-nm particle, deposition is 3-fold higher with THP compared to diffusion. To assess the ability of the systems to deliver gases to cells, ozone (O3) was used as a test agent. The reaction of O3 with an indigo dye on well surfaces showed that diffusion allowed gas-cell interaction. Increasing the flow rate in diffusion systems, when air flow is perpendicular to the cells, increases gas delivery to wells. To demonstrate this effect biologically, we used EPA's cell culture exposure system (CCES) to expose primary human bronchial epithelial cells to 0.3 parts per million (ppm) acrolein for 2 h at different flow rates. Measuring heme oxygenase-1 (HO-1) 1 h post-exposure resulted in elevated fold changes over control of 1.64, 1.82, and 4.39 at 3, 5, and 25 mL/min/well, respectively. These results show that flow rate is a critical factor that influences the biological effects. Our study shows that diffusion systems are favorable for exposures of gases, whereas systems with THP or ESP are suitable for particles. Characterization of *in vitro* exposure systems is needed prior to their use in order to understand their advantages and limitations. [Abstract does not necessarily reflect the views or policies of the US EPA.]

1430 Toxicological Investigation of Coal Fly Ash (CFA) As Soil Amendment for the Production of Bioenergy Feedstock

O. M. Awoyemi[2] and E. K. Dzantor[2]. *[1]Agricultural and Environmental Sciences, Tennessee State University, Nashville, TN and [2]Biological, Agricultural and Environmental Sciences, Tennessee State University, Nashville, TN.*

Coal fly ash has been suggested as soils amendment for production of bioenergy feedstock while reducing accumulation of the byproduct. Investigations were conducted with CFA to evaluate the suitability of the byproduct as soil amendment for production of switchgrass the model bioenergy feedstock. One experiment was performed to determine toxicological response of *Salmonella typhimurium* exposed to water extracts of CFA (0.0%, 5.0%, 10.0%, 15.0%, 20.0% and 25.0 % w/vol CFA/water), using modified Ames ISO microplate fluctuation (MPF) assay, a derivative of the traditional Ames test. The other experiments tested seed germination and growth of transplanted seedlings of switchgrass in CFA-amended Armour silt loam soil (0.0%, 5.0%, 10.0%, 15.0% and 20.0% w/w CFA/soil) using a modified soil-on-agar technique. Preliminary results of the Ames assay showed a significant ($p<0.05$) toxicity to *S. typhimurium* at 20.0 % and 25.0 % CFA after 96-hour exposure. Germination of switchgrass seeds in soil-on-agar preparations was faster when CFA was present (two days for 10.0%; 15.0% and 20.0%) than in control set-up (three days). After five days, the total number of seeds that had germinated were 4, 7, 8, 11 and 11 in 0.0%, 5.0%, 10.0%, 15.0% and 20.0% CFA amended soil-on-agar systems respectively. For transplanted seedling systems with each system containing a total of 16 seedlings, the number of seedlings that survived were 13, 13, 15, 11 and 10 in 0.0%, 5.0%, 10.0%, 15.0% and 20.0% CFA amended soil-on-agar systems respectively. 15.0% and 20.0% CFA amended soils retarded the growth of the switchgrass seedlings while a stable growth of the seedlings was observed at 10.0 % CFA even after 30 days of cultivation. These results suggest that soil amendment with CFA at rates up to 10.0% could be suitable for bioenergy feedstock production with simultaneous reduction of CFA accumulations. Studies are ongoing to assess the toxicological and molecular responses of switchgrass and potentially other bioenergy feedstock when grown in CFA-amended soils.

1431 Effect of Diesel Exhaust Exposure on the Liver in Mouse Fed with N-3 Polyunsaturated Fatty Acids-deficient Diet

M. Nakamura[4], A. A. El-ghoneimy[1,3], A. Onoda[1,4,5], Y. Shinkai[1], H. M. Shaheen[1,2], K. Takeda[1] and M. Umezawa[1]. *[1]Cent. Env. Health Next Gen., RIST, Tokyo Univ. Sci., Noda, Japan; [2]Fac. Vet. Med, Damanhour University, Damanhour, Egypt; [3]Fac. Vet. Med, South Valley University, Qena, Egypt; [4]Grad. School Pharm. Sci. Tokyo Univ. Sci., Noda, Japan and [5]JSPS Research Fellow, Tokyo, Japan.* Sponsor: K. Nohara.

Objective: Epidemiological studies have shown a positive correlation between concentration of particulate matter (PM) and fatalities related to cardiovascular disorders. Diesel exhaust (DE) is one of the main contributors of air pollutant in urban areas. Experimental studies have shown that DE exposure exacerbates non-alcoholic fatty liver disease (NAFLD)-like phenotype in diabetic mouse. These results suggest that exposure to DE containing PM may exert systemic influence and lipid metabolic system. One of the most common issues in imbalanced intake of daily diet is an absence of n-3 polyunsaturated fatty acids (n-3 PUFAs). Dietary intake of n-3 PUFAs has an anti-inflammatory effect and suppresses hepatic triglyceride (TG) accumulation. The aim of the present study was to investigate the effect of exposure to DE on mice fed with n-3 PUFAs-deficient diet. Methods: Six-week-old female C57BL/6J mice were fed control chow or n-3 PUFA-deficient diet to make CTR and n3def groups. After feeding with either of the diets for 4 weeks, the mice were exposed to clean air (CTR-CA and n3def-CA group) or diesel exhaust inhalation at 100 µg/m3, 8 hours/day, for 28 consecutive days (CTR-DE and n3def-DE group). Liver and blood samples were collected from female mice at 14-week-old. Liver histology, plasma parameters, and genes expression levels related to fatty acid synthesis were evaluated. Results: Lipid droplet accumulation was increased in the liver of the n3def group. The n-3 PUFA-deficient diet increased hepatic expression of genes related to fatty acid synthesis: Acaca, Acacb and Scd1, which promotes fatty acid synthesis. DE expose further increased the plasma leptin level and expression levels of genes related to fatty acid synthesis Acacb, Fasn and Scd1, in the liver of mice fed the n-3 PUFA-deficient diet. N-3 PUFA-deficient diet and DE exposure synergistically increased hepatic fatty acid synthesis and lipid droplet accumulation. Conclusion: The present study suggests an important mechanism for preventing toxicity of DE on the liver. DE exposure and the intake of n-3 PUFA-deficient diet may be an additional risk factor for the incidence of NAFLD.

1432 Modeling Bacterial Mixtures in the Cervix During Pregnancy and the Association with MicroRNA Expression and Subsequent Gestational Age

A. P. Sanders[3], C. Gennings[3], K. Svensson[3], A. Mercado-Garcia[4], M. Solano[4], A. A. Baccarelli[2], M. M. Tellez-Rojo[4], R. O. Wright[3] and H. H. Burris[1]. *[1]Beth Israel Deaconess Medical Center, Boston, MA; [2]Harvard School of Public Health, Boston, MA; [3]Icahn School of Medicine at Mount Sinai, New York, NY and [4]National Institute of Public Health, Cuernavaca, Mexico.*

Preterm birth affects 12% of births in the US and is associated with increased childhood mortality and morbidity. The cervix is an ideal tissue to study risk factors for preterm birth, such as bacterial vaginosis. microRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally control gene expression and play an important role in the host response to microbes. No previous studies have examined bacterially-associated alterations in cervix miRNA expression during pregnancy as biomarkers for preterm birth. We analyzed whether the association of 5 common microbes in bacterial vaginosis (*bacteroides*, *gardnerella vaginalis*, *mycoplasma*, *ureaplasma urealyticum*, and *mobiluncus*) modeled as a complex biological mixture predicted both miRNA expression and subsequent gestational age. We obtained cervical swabs from 80 women between 18 and 20 weeks gestation in the same fashion as a Pap smear. Bacterial 16S rRNA and miRNA expression were quantified using the Nanostring nCounter Analysis System. To model the candidate bacteria as a mixture, we applied a weighted quantile sum (WQS) approach and then performed linear regression to examine the associations between expression of 74 miRNAs and gestational age adjusting for women's age, parity, education, and environmental tobacco smoke. We developed a novel framework for modeling bacterial populations in the cervix as a mixture. In adjusted models, the WQS bacterial mixture was associated

with shorter gestational age (p=0.005). The bacterial mixture was also associated with altered expression of five miRNAs including miR- 494, 371a, 888, 223, and 23a (p<0.05; q<0.3). The associations with miRNA expression were largely driven by the presence of *bacteroides, gardnerella* and *mobiluncus*. Previously, we and others have shown that miR-223 expression is associated with shorter gestations. These findings suggest that subclinical bacterial colonization in the cervix might be predictive of preterm birth and that miRNAs in the cervix could be mediators of this relationship. The role of bacterial mixtures and cervical miRNA expression in pregnancy outcomes deserves further study.

1433 Safety Evaluation and Cytotoxicity of Compounds Isolated from a Bioactive Fraction of *Peltophorum africanum*, a Medicinal Plant Used to Treat Pain Associated with Inflammatory Conditions

S. A. Adebayo[1,2], J. L. Shai[1] and K. Eloff[2]. [1]*Biomedical Sciences, Tshwane University of Technology, Pretoria, South Africa and* [2]*Paraclinical Sciences, University of Pretoria, Pretoria, South Africa.*

Extracts derived from *Peltophorum africanum* (PA) is widely used in southern Africa to treat painful symptoms associated with conditions such as arthritis, infections and inflammation. However, the safety and potential cytotoxicity of the constituents of these extracts are unknown. Some medicinal plants are known to produce poisonous substances that can lead to fatal intoxication in both humans and animals. Bioassay guided fractionation of a PA leaf extract led to the extraction of a bio-active fraction with good *in vitro* anti-inflammatory activity using the anti-15 lipoxygenase (15-LOX) model. The bioactive fraction was subjected to column chromatography using silica gel, which resulted in the isolation of 7 compounds. Cytotoxicity of the isolated compounds were evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyl tetrazolium bromide (MTT) assay. The cell lines that were used included vero kidney monkey, Hep-G2 liver, and RAW 264.7 cell lines. The bioactive fraction of PA had good anti-inflammatory activity by inhibiting the activity of 15-LOX with an IC50 of 0.009±0.00 µg/mL. This was a thousand times better than the positive control quercetin (8.73±1.42 µg/mL). The safety evaluation of the isolated compounds revealed that some of the compounds are cytotoxic to the cell lines used. The results indicated that compound 3 (CP3) was relatively cytotoxic to vero cells lines (IC50=0.29 µg/mL) compared with hydrogen peroxide positive control (IC50=0.007 µg/mL). All the compounds tested had low cytotoxicity on Hep-G2 and RAW 264.7 cells compared to hydrogen peroxide positive controls (IC50=0.003 µg/mL and 4.60 µg/mL) respectively, with CP3 indicating the lowest cytotoxicity (IC50=207.88 µg/mL) on RAW 264.7 cells. Compounds with IC50≤20 µg/mL on cell lines are considered to be relatively cytotoxic. The selectivity index for the compounds ranged between 0.02 and 0.32. The structures of the isolated compounds are currently being determined. The findings in this study suggests that some of the constituents of extracts of PA could be potentially cytotoxic, with possible negative impact on the well-being of users.

1434 Health Hazard Assessment of Alcohol-To-Jet (ATJ) Synthetic Kerosene with Aromatics (SKA) Alternative Jet Fuel

D. R. Mattie[1], K. L. Mumy[3], B. A. Wong[2,3], L. M. Sweeney[2,3], G. Reddy[4], W. C. McCain[4] and T. R. Sterner[2]. [1]*711 HPW/RHDJ, Wright-Patterson AFB, OH;* [2]*HJF, Wright-Patterson AFB, OH;* [3]*NAMRU-D, Wright-Patterson AFB, OH and* [4]*USAPHC, Aberdeen Proving Ground, MD.*

Alcohol-to-jet (ATJ) Synthetic Kerosene with Aromatics (SKA) fuels are produced by dehydrating and refining alcohol feed stocks generated from non-renewable petrochemical sources or renewable fermented plant sugars. Toxicity testing was performed with two ATJ SKA formulations (each containing ≥ 70% branched paraffins and ≥ 15% aromatics) produced from lignocellulosic feedstocks, both with the addition of an operationally required JP-8 chemical additive package. Dermal irritation assays were performed using 4 hr exposures on New Zealand white rabbits. Both ATJ SKA fuels were found to be "slightly" and "moderately" irritating following semi-occluded and occluded exposures, respectively; petroleum-derived JP-8 scored similarly. In a 90-day inhalation study, male and female Fischer 344 rats (10/sex/concentration) were exposed whole body to the newer ATJ SKA formulation (0, 200, 700, or 2000 mg/m3, 6 hr/dy, 5 dy/wk, 69 exposures) in an aerosol/vapor mixture. The study resulted in a minimal alveolar hyperplasic response in the 2000 mg/m3 exposure group, and some mild behavioral changes (decreased grooming) in female rats. Additional rats (5/sex/concentration) were included in the exposures for 2 weeks to test for genotoxicity by micronucleus formation. As bone marrow cell toxicity or an increase in micronuclei were not observed, the fuel is not clastogenic. ATJ SKA fuels were tested for mutagenic potential using the reverse mutation assay (Ames test), with and without metabolic activation; no evidence of mutagenicity was found. Overall, ATJ SKA fuels were found to be similar to or less toxic than petroleum-derived JP-8. Handling of ATJ SKA fuels alone or when mixed in a 50:50 blend with petroleum-derived JP-8 is unlikely to increase human health risks in military workplaces. Occupational exposure levels for ATJ SKA fuels are recommended to remain the same as for JP-8: 200 mg/m3 (vapor) and 5 mg/m3 (aerosol). (In compliance with DODI 3216.01)

1435 Assessment of Water Cytotoxicity in Northern Alberta Rivers Near the Oil Sands

D. Y. Huang[2], I. Dey[2], L. Xie[2], W. Zhang[1], S. Gabos[3] and D. W. Kinniburgh[2,3]. [1]*Alberta Health, Edmonton, AB, Canada;* [2]*Alberta Centre for Toxicology, Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, Canada and* [3]*Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada.*

Source water monitoring is an essential environmental public health service. Water toxicity screening is of great importance to environmental managers and public health decision makers. Currently there are only a few practical bioassays that can relate environmental conditions to human health. A real-time *in vitro* cytotoxicity bioassay using the ACEA xCELLigence Real-Time Cell Analyzer system has been developed for toxicity screening in water samples at the Alberta Centre for Toxicology. This technology allows non-invasive and continuous monitoring of the perturbation of HepG2 cell growth following exposure to the combined toxic effect present in such mixtures as water samples. Several hazard indices including a water toxicity index (WTI) as well as the potential of biological effect (PoE) and cell growth inhibition (AUC) were developed to assess the cytotoxicity of source and other waters. A total of 406 river water samples were collected in northern Alberta around the oil sands region during the open water season from 2012 to 2014. The WTI ranged from 0.19 to 13.72 over the three-year period. Overall, 57% of samples had a WTI greater than the cutoff value (i.e.,1) indicating potential toxicity. Broken down by year, 96% of the sites had at least one sample being cytotoxic in 2012, with the percentages being 93% and 90% in 2013 and 2014, respectively. The minimum WTI was similar from year to year (~0.20), indicating a potential baseline value. The maximum toxicity was observed in 2012 and decreased over the years 2013 and 2014. Based on the WTI, PoE and AUC values, possible hot spots of cytotoxicity were identified. The study demonstrated the usefulness of this bioassay as a screening tool for toxicity assessment in environmental aqueous matrices, established the baseline values for the northern Alberta rivers, assessed the geographical and temporal distribution of toxicity, and identified possible hot spots for future in-depth investigation.

1436 Advancing the State-of-the-Science in Estimating Cancer Risk of Polycyclic Aromatic Hydrocarbon Mixtures

M. Pratt[5], H. Carlson-Lynch[2], L. Flowers[5], M. Gehlhaus[5], P. McClure[2], J. Melia[2], G. Rice[4], L. Teuschler[1] and K. Hogan[5]. [1]*LK Teuschler and Associates, Inc., St. Petersburg, FL;* [2]*SRC, Inc., Syracuse, NY;* [3]*Integrated Risk Information Systems, US EPA, Washington, DC;* [4]*National Center for Environmental Assessment, US EPA, Cincinnati, OH and* [5]*National Center for Environmental Assessment, US EPA, Washington, DC.*

Cancer risk assessment for exposures to mixtures of polycyclic aromatic hydrocarbons (PAHs) poses particular challenges because tumor data on specific mixtures of concern are rarely available. In lieu of mixture-specific data, one alternative has been the relative potency factor (RPF) approach, which scales the doses of each PAH in a mixture to an index chemical, i.e., benzo[a]pyrene (BaP). A limitation of this approach is the small number of carcinogenic PAHs for which RPFs have been derived (6 in 1993 EPA guidance). Commenting on the 2010 draft PAH Relative Potency Factor (RPF) Approach, EPA's Science Advisory Board (SAB) stated that an RPF approach remains the most practical approach until mixtures of concern can be tested. Being responsive to the SAB's recommendations, EPA's IRIS Program has advanced the state-of the-science for developing PAH RPFs through a systematic review of the literature reporting tumor data on individual PAHs, then focusing on bioassays with environmentally relevant routes of exposure and tumor responses ≤ 90%. Many tumor studies did not meet these criteria, or were conducted without concurrent BaP. Approaches that expand the usable database for estimating RPFs include using earlier time points and, in the absence of BaP, using concurrently tested PAHs as intermediate index chemicals. Characterization of uncertainty includes quantification of

variability across studies and sensitivity analyses to evaluate the impact of data choices (e.g., incidence vs. multiplicity) and modeling strategies. Despite the use of these innovative approaches, there remains a critical need for data that are better suited for the estimation of cancer risk from exposure to PAHs, both for the more practical RPF approach in the near term and, ultimately, for whole PAH mixtures. (The views expressed are those of the authors and do not necessarily reflect the views or policies of the US EPA).

1437 Mixtures of Full and Partial Agonists: Several Mechanisms of Receptor Activation Are Consistent with Generalized Concentration Addition

T. F. Webster. *Department of Environmental Health, Boston University School of Public Health, Boston, MA.*

Concentration addition (CA) is conventionally used to predict the effect of mixtures that act by similar mechanisms or modes of action. But CA cannot make predictions for effect levels above that achievable by the least efficacious compound in the mixture. As partial agonists are common, we proposed generalized concentration addition (GCA). GCA requires specification of mathematical function for individual dose-response curves; these functions must be invertible. We have used knowledge of mechanism to construct biologically based models and successfully tested mixture results against empirical data. A key part of the model involves the step of receptor activation, allowing differences in efficacy. Biological mechanisms include changes in receptor conformation, binding of cofactors etc. Several models are commonly used. The del Castillo-Katz model assumes that the ligand-receptor complex and its activated form are in equilibrium (e.g., two conformations), e.g., A + R ~ AR ~ AR*, where A is an agonist, R is the receptor and AR* is the activated receptor-ligand complex. The well-known operational model of pharmacology assumes a specific functional form linking AR and AR*. The full two stage model also allows the activated ligand-receptor complex to form via two pathways, from AR and A+R*. In this paper we show that if mixtures are GCA at the step of receptor binding, then they will also be GCA following activation by all three activation models. Thus, these similar biological mechanisms do not change the fundamentally additive nature of receptor-ligand interaction.

1438 DNA Strand Breaks Induced by Organic Extracts from Engine Emissions Produced at Various Engine Operating Modes Using Diesel or Biodiesel (B100) Fuel

B. Novotna[4], J. Stolcpartova[3,4], V. Beranek[1], M. Vojtisek-Lom[1,2] and J. Topinka[4]. *[1]Center of Vehicles for Sustainable Mobility, Czech Technical University in Prague, Czech Republic; [2]Faculty of Mechanical Engineering, Technical University of Liberec, Czech Republic; [3]Institute of Environmental Studies, Charles University in Prague, Czech Republic and [4]Institute of Experimental Medicine AS CR, Prague, Czech Republic.*

High amount of traffic emissions originates from a relatively low number of vehicles and relatively short time intervals of the engine operation. Hence, the analysis of the toxic effects of emissions should reflect real traffic conditions. We aimed to detect genotoxicity of organic extracts from particulate matter in the exhaust of diesel engine Zetor 1505 running on diesel and biodiesel (B100) fuels at various operating modes typical for transit truck traffic in large cities, i.e. the short idle (first 5 min of idle), extended idle (20-80 min), full load after idle, full load stabilized and 30% load. Diluted exhaust was sampled with high volume samplers on Teflon coated filters. Filters were extracted with dichloromethane and DNA damage was analyzed using comet assay with the inclusion of formamidopyrimidine DNA glycosylase and endonuclease III to recognize oxidized DNA bases, respectively. A549 cells were exposed to extractable organic matter (EOM) for 4 and 24 hours at dose corresponding to 0.001 m3 of undiluted exhaust gas per ml cell media. At 4 h exposure interval, all samples from B100 and diesel emissions induced in cells a significant DNA damage. EOM from extended idle engine mode exerted the strongest effect. Twenty hours later, the cells exposed to diesel EOM exhibited further increase of DNA strand breaks compared to preceding interval. In contrast, DNA damage seemed to be fully repaired in cells treated with B100 EOM, except of those exposed to exhaust from the extended idle engine mode. The results suggest that (1) the diesel emissions are more genotoxic than the emissions from B100 and (2) the different compounds play a key role in genotoxicity of diesel and B100 emissions. Support: EC (LIFE10 -ENV-CZ-651) and Czech Science Foundation (13-01438S).

1439 Use of Partial Least Squares Regression to Identify GC/MS Spectral Peaks Correlated with Toxicity Outcomes in Rats of Crude Oil from Various Sources

J. T. Salley[5], R. J. Al Suliman[5], S. Jaligama[2], H. Barnett[5], S. Korpela[1], V. Y. Hebert[3], T. R. Dugas[1] and S. A. Meyer[5]. *[1]Comparative Biomed. Sci., Louisiana State Univ. Sch. Vet. Med., Baton Rouge, LA; [2]Pediatrics, Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN; [3]Pharmacol., Toxicol. & Neurosci., Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA; [4]Sch. Sciences, University of Louisiana-Monroe, Monroe, LA and [5]Toxicology, University of Louisiana-Monroe, Monroe, LA.*

Anticipated health effects of crude oil are generalized from those associated with prior releases, although compositional differences from different sources indicate that effects would vary. Here, we determined ranges of values for several toxic effects of acute exposure to LA sweet, Nigerian sweet, Iraq high sulfur crude oils (ONTA, Inc., Toronto), and Deepwater Horizon (DWH) crude oil samples A010G4 "Surrogate", A0083Q "MASS Aug 15" and A001EP/EQ "SOB MAY 22" (AECOM, Inc., Fort Collins, CO). Female Sprague-Dawley rats treated with 2 daily doses (2.5, 5 ml/kg, p.o.) were bled 48h later for hematology, clinical chemistry and serum 8-isoprostane (8-IsoP), the latter a biomarker for oxidative stress. Rats treated with all oils had elevated serum alkaline phosphatase and relative liver weights. Relative spleen weights decreased for high dose Nigerian, Iraqi, EQ and 3Q. Granulocytes increased with high dose of Iraqi and DWH oils and 8-isoP increased with all but Nigerian and Iraqi oils. To test whether partial least squares (PLS) regression can identify chemical constituents *in situ* within crude oil mixtures active in mediating a given toxic effect, we regressed peak areas of chromatograms from GC/MS (HP-5 column) on serum 8-isoP and spleen weights. Among 80 peaks with non-negligible range, PLS (NIPALS algorithm) gave highest weighting for n-heptane and toluene for the first latent factor fitted to 8-isoP response. An isomer of trimethylbenzene was most influential for spleen weight. These data emphasize that the toxic profile for any given crude oil is unique and demonstrate the feasibility of predicting a crude oil's toxic effects based upon multivariate regression of content of constituents from analytical chemical fingerprints.

1440 Strengths and Limitations of Generalized Concentration Addition in Modeling PPARγ Activation by Endocrine-Disrupting Compounds

J. Watt, T. Webster and J. Schlezinger. *Environmental Health, Boston University School of Public Health, Boston, MA.*

Current high throughput initiatives such as EPA's ToxCast and Tox21 address the problem of testing the toxic potential of thousands of individual environmental compounds. However, predicting toxicity of chemical mixtures represents a large-scale problem in need of practical analysis tools. In the case of endocrine disrupting chemicals, low dose co-exposures have the potential for significant, additive effects at the molecular level. We previously developed a Generalized Concentration Addition (GCA) model to predict additive mixture effects of aryl hydrocarbon receptor agonists with varying efficacies and potencies. In the current study, we tested the validity of the GCA approach to predict the activation of a nuclear receptor by dual and complex mixtures of ligands. PPARγ is a primary target of adipogenic endocrine disrupting compounds that heterodimerizes with the retinoid-x receptor alpha (RXRα). Using a PPRE-dependent luciferase reporter assay similar to assays used in ToxCast, we generated empirical data of human PPARγ activation by a binary combination of the PPARγ reference compound (and full agonist) rosiglitazone and a non-thiazolidinedione partial agonist. We constructed a GCA response surface from the individual chemical dose-response data and show it to be a reasonable approximation of the experimental data. Then, we applied the GCA approach to model a complex mixture of active doses of five phthalates (including parent compounds and metabolites), which were chosen based on their identification as PPARγ agonists by ToxCast data. We further tested GCA as a model for predicting additive effects of specific RXRα agonist (e.g. LG100268), dual RXRα/PPARγ agonist (e.g. tributytin) and PPARγ agonist pairs. Our results support the use of Generalized Concentration Addition for modeling nuclear receptor activation by complex mixtures, and we identify advantages and caveats to the use of component-based additive models by regulatory agencies and risk assessors.

1441 *In Vitro* Toxicological Assessment of 4-Methylcyclohexane Methanol and Propylene Glycol Phenyl Ether Mixtures

E. Fabyanic, A. Han, M. Prediger and J. Boyd. *Bennett Department of Chemistry, West Virginia University, Morgantown, WV*. Sponsor: J. Boyd.

On January 9, 2014, thousands of gallons of an industrial chemical, crude 4-methylcyclohexane methanol (MCHM), and a secondary solvent of propylene glycol phenyl ether (PPH), leaked from industrial tanks into the Elk River in Charleston, West Virginia. Consequently, an estimated 300,000 residents were reported to exhibit symptoms of MCHM exposure and were advised not to use or drink water. Unfortunately, the limited toxicological data on MCHM and PPH were insufficient for appropriate evaluation of the health risks. While the extent of chemical exposure is uncertain, the observed symptoms of MCHM exposure, including nausea, skin irritations, headaches, and dizziness indicated a possible threat of a toxic substance. In this preliminary study, we assessed the extent of plasma membrane degradation after 24 hour exposures to varying concentrations (0-1000 µM) of the two mentioned chemicals equivalent to or associated with ones involved in the spill. While PPH was not a major part of the spill, the presence of a secondary chemical nonetheless generated concern for the public's health. Evaluation of four different cell lines, HEK 293 (kidney), HepG2 (liver), H9C2 (heart), and GT17 (brain), provided some insight regarding altered cellular responses observed in four varying tissue systems. Cell death was not induced for any cell lines except at the highest dose of MCHM (1000 µM) and PPH remained ineffective across all doses for all cell lines. In addition to evaluating the toxicity of single exposures of the two chemicals, cellular response was evaluated after simultaneous co-exposure of both chemicals. Even though PPH alone did not elicit any changes, indications of additive toxicity were observed in HEK293 cells. The presence of both MCHM and PPH increased lethality across all MCHM and PPH doses in GT17 and H9C2 cells. HEPG2 cells were only vulnerable when exposed to 1000 µM in conjunction with all doses of PPH. These findings indicate the significance of examining co-exposures in order to fully understand the potential toxic effects.

1442 An *In Vitro* Absorption Screen with Rosin/Resins Using Everted Rat Intestinal Sacs

L. Higgins[1], R. Powrie[1], M. Penman[2], S. Green[2], R. Lobbes[2], L. Rodenburg[2] and C. Elcombe[1]. [1]*CXR Biosciences, Dundee, United Kingdom and* [2]*Hydrocarbon Resins & Rosin Resins REACH Consortium (H4R), Brussels, Belgium.*

The aim of this study was to determine the potential intestinal absorption of a series of 32 rosin and rosin derivatives (rosin, its esters, adducts and esters of adducts) using everted rat intestinal sacs. Everted sacs were incubated in Fed-State Simulated Intestinal Fluid (FeSSIF) saturated with individual rosin/resin, acid/adducts and esters (dissolved in ethyl acetate) at 37°C for 1 hour after which time the sac contents were collected, extracted and analysed for mass spectra fingerprints (LC/MS) in electrospray negative ion mode between 500-5000 m/z. These fingerprints were compared to mass spectra fingerprint for each individual rosin/resin acids/adducts and esters extracted from FeSSIF to give a fingerprint of moieties capable of being absorbed from the incubation medium to the serosal side of the intestinal sacs. The mass spectra fingerprint of incubation mixture for each substance shows a set of complex substances with all moieties present, e.g. isomers of mono-, di- and tri-esters. The mass fingerprints of rosin/resin acid/adducts and esters identified in the serosal fluid of the intestinal sac was less complex than the incubation mixture. For each substance, rosin, ester or adduct salts the mass spectrum consisted of very similar clusters of ions predominantly in the 300, 600 and 900 m/z ranges. These are consistent with isomers of rosin, rosin dimers, and rosin trimers. Analysis suggests that the absorption of higher molecular weight components does not occur. Comparison of spectra between the intestinal sac serosal and incubation medium for rosin esters and adduct salts suggests only the free acids are being transferred across the gut membrane.

1443 *In Vitro* Hepatotoxicity of Mixtures of Benzylpiperazine (BZP) and 3-Trifluoromethylphenylpiperazine (TFMPP)

D. Dias da Silva, P. Moreira, M. J. Silva and H. Carmo. *Laboratory of Toxicology, UCBIO/Requimte, University of Porto, Porto, Portugal.* Sponsor: S. Ali.

In recent years, seized ecstasy tablets showed not to contain 3,4-methylenedioxymethamphetamine (MDMA), but to be instead comprised of phenylpiperazine derivatives benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP). Our work compared the *in vitro* toxicity of BZP and TFMPP combinations in two different cellular models, HepaRG cells and primary rat hepatocytes. The cells were exposed to the drugs and the viability assessed by the MTT assay, at 37oC, after 24 hours. Based on the concentration-response curves for each component, mixture effect expectations were computed using the independent action and concentration addition (CA) models. Three different combinations were tested at different drug ratios (BZP 2: TFMPP 1; BZP EC50: TFMPP EC50 and BZP EC01: TFMPP EC01). The experimental outputs of the mixtures were compared with the additivity predictions. Stress endpoints further evaluated included quantification of ROS/RNS, GSH/GSSG, ATP and mitochondrial membrane potential changes. The metabolic profile of single/combined drugs was characterized by GC-MS. Primary rat hepatocytes were more sensitive than HepaRG cells to BZP toxicity (EC50 of 2.15 mM and 6.6 mM, respectively) and TFMPP (EC50 of 0.13 mM and 0.46 mM, respectively). CA was the most appropriate model to predict the mixture. Substantial mixture effects occured when each drug was present at concentrations that individually produced no effects. The metabolic profiling of single/combined drugs suggests that the co-exposure of BZP with TFMPP improved metabolism of both drugs, particularly of BZP. Also, changes in some oxidative stress by the combination of BZP and TFMPP were noted namely, an increase of RNS/ROS production, decrease of GSH, ATP depletion and mitochondrial impairment. Concluding, the present work clearly demonstrated that potentially harmful interactions among BZP and TFMPP are expected when these drugs are taken concomitantly. This work received financial support from the EU FEDER funds - COMPETE and FCT - PIDDAC through projects Pest-C/EQB/LA0006/2013 and INCENTIVO-LA6-2014 (grant INCENTIVO/EQB/LA0006/2014), from the EU FEDER funds under the framework of QREN through Projects NORTE-07-0124-FEDER-000066 and NORTE-07-0124-FEDER-000067.

1444 A Mechanistic Comparison of Human and Mouse Lung Cell Responses to a Polycyclic Aromatic Hydrocarbon Mixture: Inflammation and the P38 Pathway

R. S. Osgood[3], K. Velmurugan[3], K.-N. Xiong[3], J. Xiong[3], B. L. Upham[2] and A. K. Bauer[3]. [1]*Environmental and Occupational Health, University of Colorado Anschutz Medical Campus, Aurora, CO;* [2]*Pediatrics and Human Development, Michigan State University, East Lansing, MI and* [3]*Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO.*

Low molecular weight polycyclic aromatic hydrocarbons (PAHs) are more prevalent in the environment, occupational settings, as well as in secondhand smoke, when compared to the high molecular weight genotoxic species like B[a]P. They are typically considered non-genotoxic and little is known about their inflammatory effects in the lung. Secondhand smoke is a complex mixture and to model this feature *in vitro* utilizing both mouse and human cells, we examined the additive and synergistic effects of a binary exposure of two secondhand smoke LMW PAHs (1-methylanthracene (1-MeA) and fluoranthene (Flthn)). We tested single PAH and a binary mixture for the ability to dysregulate gap junction intercellular communication (GJIC; scalpel-loaded/dye transfer assays (GJIC)), activate mitogen activated protein kinase (MAPK) P38 (immunoblotting), and induce transcriptional changes (Affymetrix Mouse Gene 2.0 ST array, qRT-PCR) in C10 cells, a mouse, non-tumorigenic alveolar type II cell line, and two normal human bronchial epithelial cell lines, BEAS2B and HBE-1. Both 1-MeA and Flthn were able to dysregulate GJIC in all three cell lines while displaying an additive effect when used in a mixture. P38 MAPK was rapidly phosphorylated by both PAHs and inhibition of this pathway reversed dysregulation of GJIC. A mixture verses single PAH exposure produced a synergistic effect on gene expression and inflammatory mediators which was reduced by p38 inhibition in a subset of genes. Our results demonstrate similar responses in both mouse and human lung cells in PAH mixtures that are more relevant to real world exposures and could uncover common pathways of LMW PAH toxicity between mouse and human lung cells to improve our mechanistic knowledge of how these compounds potentially influence inflammatory lung diseases.

1445 QSAR Modeling Toxicity Predictions of the Constituents of Crude 4-Methylcyclohexanemethanol (MCHM) and Structurally Related Chemicals

B. Winans[1], R. M. Novick[1], S. M. Green[2] and D. J. Paustenbach[1]. [1]*Cardno ChemRisk, San Francisco, CA and* [2]*Eastman Chemical Company, Kingsport, TN.*

On January 9, 2014, approximately 10,000 gallons of a mixture containing crude 4-methylcyclohexane methanol (MCHM) were spilled into the Elk River in Charleston, West Virginia. Crude MCHM is a mixture of a number of chemicals, including MCHM, 4-(methoxymethyl)cyclohexanemethanol, methyl 4-methylcyclohexanecarboxylate, dimethyl 1,4-cyclohexanedicarboxylate and 1,4-cyclohexanedimethanol. This family of chemicals has been well studied; however, some data gaps existed in 2014. Quantitative structure-activity-relationship ((Q)SAR) modeling and structural alerts were used to predict the toxicity of the constituents of crude MCHM, as well as a number of structurally related chemicals, including 1,4-cyclohexanedicarboxylic acid, 2-methylcyclohexanemethanol, 4-methylcyclohexanecarboxylic acid, 4-[(ethenyloxy)methyl]-cyclohexanemethanol and alpha,alpha,4-trimethyl-cyclohexanemethanol. The constituents of crude MCHM and the structurally related chemicals were predicted to have low to moderate acute toxicity, low potential for skin and eye irritation and low mutagenic potential, which agreed with the available experimental data in animals. Some of the chemicals in this group were also predicted to have the potential for skin sensitization and developmental toxicity, but the animal data did not fully support the predictions. Specifically, for some chemicals, rodent, zebrafish and nematode studies of these endpoints did not agree with the predicted outcome or exhibited mixed results across assays. The inconsistency between predicted and experimental values from testing in rodents for skin sensitization and developmental toxicity suggests that these models may not be valid for predicting these adverse effects for this group of chemicals. Predictions from (Q)SAR modeling and available experimental data for the constituents of crude MCHM and structurally related chemicals suggest that these chemicals pose little risk to human health at the concentrations likely experienced following the Elk River spill.

1446 Co-Exposure to Iron, Lead and Cadmium Increases Susceptibility to Cardiovascular Disease and Hepatic Damage in Male Wistar Rats

O. A. Odunola[2], O. A. Akinbode[2], M. A. Gbadegesin[2], S. E. Owumi[2], K. A. Akinwumi[1], A. M. Adegoke[2] and O. J. Olugbami[2]. [1]*Biochemistry Department, Bells University, Ota, Nigeria and* [2]*Biochemistry Department, University of Ibadan, Ibadan, Nigeria.*

Bio-accumulation of heavy metals has been a cause of great cellular burden as their intoxication can damage vital organs including lungs, kidney, liver and bones. Chronic exposures to certain heavy metals have also been associated with increased risk of cancer. Co-exposure of man and animals to these metals beyond permissive environmental levels seems unavoidable especially in developing countries. We therefore, report here the effects of co-exposure to lead (Pb), cadmium (Cd) and iron (Fe) in wistar rats. Forty male rats divided into eight groups of five animals each were treated as follows for 4 weeks: Group 1 (negative control) distilled water only. Groups 2, 3 and 4 (Pb; 22.5mg/kg bd wt.), (Fe; 70mg/kg bd wt.) and (Cd; 3.2mg/kg bd wt.) respectively. Group 5, Pb; 22.5mg/kg + Fe; 70mg/kg , Group 6, Fe; 70mg/kg + Cd; 22.5mg/kg, Group 7, Cd; 3.2mg/kg + Pb; 22.5mg/kg, Group 8, Pb; 22.5mg/kg + Cd; 3.2mg/kg + 70mg/kg. Liver function and lipid profile were analyzed using serum of treated animals. The enzymatic activities of aspartate amino transferase (AST) of Groups 4, 6, 7 and 8 increased significantly ($p< 0.05$) as compared to the negative control group. Also, Group 8 animals exposed to the three metals showed significant ($p<0.05$) increase in gamma glutamyl transferase (ΓGT) activities as compared with the negative and Groups 2, 3 and 4. Similarly, the serum concentration of LDL-Cholesterol of Group 8 significantly increased ($p<0.05$) in Group 8 as compared to Group 1. The levels of HDL-Cholesterol and triglycerides of group administered the three metals decreases but were not significant as compared with Group 1. Histopathological examination of liver sections showed no visible lesions in the negative control and the group administered Fe only. Groups administered Pb and Cd only showed severe periportal infiltration by mononuclear cells while co-administration with the three metals showed a mild to moderate periportal infiltration by mononuclear cells. The co-administration of Iron, Lead, and Cadmium may therefore increases susceptibility to cardiovascular disease and hepatic damage and much research effort should be directed towards minimizing co-exposure to heavy metals, remediation and their effect *in vivo* and prophylaxis.

1447 The Effects of Surinamese Nutraceutical and Medicinal Plant Extracts on Scratch Wound Healing of Endothelial Cells

Y. Asare[2], D. Mans[1] and J. Wickliffe[2]. [1]*Antom De Kom University, Paramaribo, Suriname and* [2]*Global Environmental Health Sciences, Tulane University, New Orleans, LA.*

Aqueous extracts were prepared from several plant species from Suriname to assess their effects on the proliferation and migration of cells used in the scratch wound healing assay. Plant species were selected based on their uses in traditional medicine and as nutriceuticals. Primary human umbilical vein endothelial cells, HUVECs, were cultured in 96-well plates. When the cells reached approximately 80% confluence, they were mechanically scratched using a 96-well wounding pin replicator (V&P Scientific, San Diego, California). Specific wells were supplemented with either base medium, complete medium, or complete medium plus defined concentrations of each extract. At 16-18 hours post-treatment, cells were fixed, dried, and stained with sulforhodamine B for visualization. The wound areas were measured and the level of closure of the scratches in wells with plant extracts were compared to wells with base or complete medium to assess any "healing" effects of the extracts. We have thus far observed that extracts from *Aloe vera* (L.) Burm.f. and *Eugenia uniflora* L. appear to promote wound closure under our experimental conditions. The results of this experimental series may provide insight into the possible biomedical properties of certain extracts and be used to guide further analytical chemical characterization of such extracts.

1448 Two Simulated-Smog Atmospheres With Different Chemical Compositions Produce Contrasting Mutagenicity in *Salmonella*

J. Zavala[4], J. Krug[2], S. H. Warren[3], Q. T. Krantz[3], C. King[3], S. H. Gavett[3], M. Lewandowski[2], W. A. Lonneman[2], T. E. Kleindienst[2], M. Meier[1], M. Higuchi[3], M. I. Gilmour[3] and D. M. DeMarini[3]. [1]*Health Canada, Ottawa, ON, Canada;* [2]*NERL, US EPA, Research Triangle Park, NC;* [3]*NHEERL, US EPA, Research Triangle Park, NC and* [4]*ORISE, NHEERL, US EPA, Research Triangle Park, NC.*

Ozone (O3), particulate matter (PM), and nitrogen dioxide (NO2) are criteria pollutants used to evaluate air quality. Using a 14.3-m3 Teflon-lined smog chamber with 120 UV bulbs to simulate solar radiation, we generated 2 simulated-smog atmospheres (SSA-1 & SSA-2) with different concentrations of these criteria pollutants to explore their mutagenicity. For SSA-1 (high PM, low O3/NO2), we added 6 parts per million carbon (ppmC) α-pinene, 24 ppmC gasoline, and 0.5 ppm nitric oxide (NO) continuously into the chamber with nebulized ammonium sulfate (2 µg/m3) to provide a nucleation base for secondary reaction products. The photo-oxidized atmosphere produced 97 ppb O3, 244 ppb NO2, and 1.07 mg/m3 PM2.5. For SSA-2 (low PM, high O3/NO2), we added 6 ppmC isoprene, 9 ppmC gasoline, and 0.9 ppm NO, which produced an atmosphere with 440 ppb O3, 586 ppb NO2, and 55 µg/m3 PM2.5. We measured the concentrations of aromatics, peroxyacetyl nitrate (PAN), paraffins, olefins, and other volatile organics produced as a result of photochemical reactions. Plates of Salmonella TA100 ± S9 spread in a top agar were exposed for 0-14 h, incubated for 72 h, and colonies were counted. Both SSA-1 and -2 were mutagenic only with the lights on and did not require S9. Thus, all the mutagenicity was direct-acting and did not require metabolism. SSA-2 was ~3 times more mutagenic than SSA-1, and unlike SSA-1, SSA-2 was cytotoxic past 3 h of exposure. O3 at 440 ppb was not cytotoxic or mutagenic; therefore, the observed results with SSA-2 were due only to other secondary reaction products. Using next-gen DNA sequencing, the SSA-1-induced mutants were 50% G to T and 50% G to A. This study shows that secondary reaction products, which are typically not measured, play a key role in air-pollution mutagenicity. [Abstract does not necessarily reflect the views or policies of the US EPA.]

1449 Abstract Withdrawn by Author.

1450 In Search of the Proinflammatory Constituents of Combustion-Derived Particulate Matter

J. Øvrevik[2], A. I. Totlandsdal[2], A. Kubátová[1], N. Bach[2], A. K. Bølling[2], R. E. Cochran[1], K. Ondrušová[1], B. C. Brinchmann[2], E. le Ferrec[3], D. Lagadic-Gossmann[3], P. E. Schwarze[2], J. A. Holme[2] and M. Låg[2]. *[1]Chemistry Department, University of North Dakota, Grand Forks, ND; [2]Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway and [3]UMR Inserm U1085/IRSET, University of Rennes 1, Rennes, France.* Sponsor: U. C. Nygaard.

The toxicity of combustion particles such as diesel exhaust (DEP) and wood smoke (WSP) has been attributed to the complex mixture of adhered organic chemicals (OCs). However, the active substances remain to be identified. OCs were extracted from DEP and WSP by methanol washing and induction of cytotoxicity and proinflammatory cytokines (IL-6 and IL-8) were examined in human bronchial epithelial cells (BEAS-2B). Methanol extractable (ME) organics accounted for the majority of toxicity induced by DEP. Fractionation of DEP-/WSP-ME by solid phase extraction (SPE) revealed that high-polar soluble constituents caused the majority of the cytotoxicity from DEP and WSP and contributed to IL-6 responses. Non-polar soluble constituents had no effect on cytotoxicity but contributed to both IL-6 and IL-8 responses. The effects of non-polar constituents appeared to involve redox-regulated responses, while high-polar constituents induced their effects through redox independent mechanisms. Inhibition or knock-down of the aryl hydrocarbon receptor (AhR) did not attenuate DEP-induced IL-6/-8 responses and an inverse relationship between induction of CYP1A1 and cytotoxicity/cytokines were observed. Analysis of >120 polycyclic aromatic hydrocarbons (PAHs) revealed that these constituted <1% of the total extractable organic carbon, and the PAH-distribution did not correlate with toxicological effects of the SPE-fractions. The toxicity of DEP and WSP could not be attributed to the occurrence of PAHs. Moreover, classical AhR-ligands such as BaP and dioxin-like compounds did not appear to be the main drivers of cytotoxicity or cytokines induced by DEP and WSP. DEP- and WSP-extracts are now further characterized for the content of other major classes of compounds including alkanes, carboxylic acids, aldehydes and high molecular weight species, and tested for biological effects in different cell models.

1451 *In Vitro* Screening to *In Vivo* Testing of Polycyclic Aromatic Compounds at the National Toxicology Program

C. V. Rider, S. S. Auerbach, J.-H. Hsieh, E. Tokar, D. Germolec, S. S. Ferguson, K. Shockley, E. Mutlu, S. Masten and S. Waidyanatha. *NTP/NIEHS, Research Triangle Park, NC.*

Polycyclic aromatic compounds (PACs) are ubiquitous environmental contaminants from fossil fuels and combustion of organic material. PACs include a broad range of structurally diverse constituents that exist in complex and dynamic mixtures. In response to deficiencies in available data for assessing human health risk associated with exposure to PACs, the National Toxicology Program has developed a research plan that includes three major efforts: 1) chemical analysis of PACs in complex environmental mixtures to inform exposure understanding, 2) toxicity characterization of a wide range of PACs using *in vitro*, alternative animal, and short-term rodent studies, and 3) comparison of the available approaches for predicting risk associated with exposure to PAC mixtures. Ongoing exposure characterization of samples (e.g., source oil, weathered oil) indicates that substituted PACs can be present at higher levels than parent compounds. Efforts to characterize the toxicity of PACs include analysis of data from the Tox21 high throughput screening program, development of novel *in vitro* assays to generate high content data, and 28-day immunotoxicity studies in mice. Using a cheminformatics approach based on physicochemical characteristics, 225 PACs were identified in the Tox21 10K library. Data for these PACs from 20 receptor-based and stress response assays were extracted and evaluated. The assays that were most informative for clustering the PACs into activity-based groups were: aryl hydrocarbon receptor agonism, mitochondrial membrane potential inhibition, retinol signaling pathway, estrogen receptor agonism, and glucocorticoid receptor antagonism. A subset of 25 PACs were assessed for cytotoxicity in 4 cell lines (MCF7, PC3, HL60, HepG2) and 9 PACs were further evaluated for gene expression changes in HepG2 cells. Finally, 28-day immunotoxicity studies in mice with the reference PACs benzo[a]pyrene and phenanthrene, indicated a wide range of immune suppression, with EC50s of 6 mg/kg/d and > 200 mg/kg/d, respectively. In comparing *in vitro* and short-term *in vivo* results to available *in vivo* cancer data, we found that positive responses in these assays were generally associated with higher carcinogenic potency PACs. Future work will be aimed at evaluating mixture predictions based on component and whole mixture data.

1452 An Exposure Profile Approach to Investigate Health Risks Associated with Chemical Mixtures

E. S. Coker, B. Woods, H. Krapfl and B. Malczewska-Toth. *Environmental Health Epidemiology Bureau, New Mexico Department of Health, Santa Fe, NM.*

Humans are exposed to a variety of chemicals in the environment, which presents an emerging and core issue in toxicology. While typical toxicological analyses seek to determine health risks from chemical mixtures by means of strict dose-response relationships or chemical interactions, such an approach may not properly characterize health risks associated with environmental chemical mixtures due to the influence of other non-chemical environmental factors. Also, no single statistical approach has been put forth to clarify the health risks associated with environmental chemical mixtures since such data tend to exhibit high dimensionality and a high degree of correlation between covariates, which challenges traditional regression models. We implement a dimension reduction clustering technique, known as Bayesian profile regression, to evaluate the statistical association between air pollution mixtures (PM2.5, Ozone, SO2, and NO2) with asthma emergency department (ED) visits in New Mexico. We further incorporate environmental factors such as temperature and seasonality to assess whether multi-pollutant health risks differ across such co-factors. We found that days characterized by high PM2.5 and Ozone exposure showed the greatest risk of asthma ED visits, whereas days characterized by elevated SO2 and NO2 showed lower risk. Days with low exposures for all pollutants showed the lowest risk of ED visits. Moreover, days with elevated daily temperatures during summer exacerbated asthma risks associated with high PM2.5 and Ozone exposure days. While our study is rooted in environmental epidemiology, our findings have important implications for toxicologists. Firstly, toxicology research can benefit from exposure profile clustering techniques since the environmental chemical mixtures that people are exposed to will typically exhibit an exposure profile that can be modeled using our statistical approach. Secondly, our study implies that environmental co-factors, such as temperature and season, should be considered by toxicologists when performing toxicological inquiry in order to better characterize the health risks from environmental chemical mixtures.

1453 Feasibility of Using Disability-Adjusted Life Years (DALYs) in Human Health Risk Assessment from Exposure to Hazardous Air Pollutant (HAP) Mixtures

M. Mumtaz[2] and Y. Zhou[1]. *[1]Division of Environmental Hazards and Health Effects, NCEH/CDC, Atlanta, GA and [2]Division of Toxicology and Human Health Sciences, ATSDR/CDC, Atlanta, GA.*

Risk assessment, in practice, often involves analyzing specific health effects of single chemicals and individually determining their impact on human health. However, realistic human exposures are much more complex. To evaluate mixtures within their regulatory power and respective jurisdictions, various federal agencies have developed guidance that includes three basic data driven approaches, with some variations. The hazard index (HI), a bottom-up approach, is most often used wherein individual chemical effects are first calculated and then aggregated to assess the mixtures of interest. We studied the use of Disability-Adjusted Life Years (DALYs) to estimate carcinogenic effects in human populations from exposure to mixtures of hazardous air pollutants (HAPs) that are most frequently encountered in the environment. The National-Scale Air Toxics Assessment (NATA) database was analyzed to identify HAPs with cancer risk greater than one in a million in > 5% of the census tracts across the United States. The most frequently found HAPs were formaldehyde, carbon tetrachloride, acetaldehyde, and benzene and their combinations. We then calculated their health impact by converting lifetime cancer risk reported in the NATA database to years of healthy life lost or DALYs in both urban and rural areas. The cumulative annual cancer risk of the top 10 carcinogenic air toxics was about 1,600 DALYs in the United States or 0.6 DALYs per 100,000 people. Formaldehyde and benzene together contributed to about 60 percent of the total cancer risk. Our findings reveal that, of the many HAPs, only a few affect public health significantly at the national level. Future analysis will focus on systemic health effects from exposure to these mixtures.

1454 Toxicological-based Classification of Petroleum Hydrocarbon Fractions for Assessment of Air Concentrations

G. Balagopal, O. Grabovska, D. Piche, D. Jugloff, T. Onica and J. Schroeder. *Human Toxicology and Air Standards, Ontario Ministry of the Environment and Climate Change (MOECC), Toronto, ON, Canada.*

Petroleum hydrocarbons (PHCs) derived from crude oil exist as complex mixtures of aromatic and aliphatic compounds of varying carbon numbers. The MOECC has developed a new approach for assessing groups of PHCs released to air using toxicologically-based classifications. This approach differs from existing approaches used primarily to assess soil concentrations, which are based on physicochemical properties, carbon number and aromatic/aliphatic grouping. The MOECC approach includes a new classification of PHC fractions based on toxicodynamic (TD) (critical endpoint) and toxicokinetic (TK) principles and parameters (for example: blood: air/tissue: blood partition coefficients and intrinsic clearance-Vmax/Km), along with suggested surrogates and indicators for each fraction. Based on the TD and TK analysis within the range of volatile/semi-volatile PHCs, the following toxicological fractions have been grouped for assessment: Aliphatic C5-8, aliphatic C9-16 and aromatic C9-12. For these fractions, the concentrations of all PHCs are summed (with certain exceptions based on toxicity) and the total concentration is compared to a standard for a surrogate PHC. Surrogates proposed are n-hexane, jet propellant 8, and trimethylbenzene for the aliphatic C5-8, aliphatic C9-16 and aromatic C9-12, respectively. This approach has allowed the toxicological assessment of PHCs released to air either as a group or as an individual chemical for which limited toxicological data exists.

1455 Optimal Experimental Design and Statistical Modeling for Identifying Toxicological Interactions of Environmental Pollutants

J. T. Perkins, M. Petriello, L. Xu, B. Hennig and A. Stromberg. *Department of Statistics, University of Kentucky, Lexington, KY; Superfund Research Center, University of Kentucky, Lexington, KY.*

Exposure to persistent environmental pollutants is a risk factor for the development or progression of many chronic inflammatory diseases, including cardiovascular diseases. Due to multiple experimental and statistical challenges, the studies of molecular toxicology and risk assessment have traditionally focused on dose response relationships of a single toxicant or a binary mixture. However, actual human exposures are complicated and encompass mixtures of pollutants, metals, and other poorly defined stressors. Thus, to determine the risk related to exposure to environmental pollutants we developed a multi-factorial experimental design coupled with a statistical model capable of identifying synergistic and antagonistic toxicant effects. In addition, we created an open source statistical analysis program capable of performing the appropriate calculations. To validate the statistical model, vascular endothelial cells were exposed to tertiary, binary, and single congener combinations of environmentally relevant polychlorinated biphenyl mixtures and expression of inflammatory genes were determined via qPCR. The resulting data were analyzed using a statistical model designed to identify interactions within the toxicant mixtures, which would not have been otherwise elucidated. The experimental design and statistical model used within this study proved to be an effective tool for identifying interactions between environmentally relevant pollutant mixtures and biomarkers of disease risk. The open source software that we developed will enable others to perform similar analyses, which will hopefully lead to increased research into mixtures of toxicants.

1456 The Effect of Green Tea Extract on Working Memory in Healthy Women

Y. Liu, A. D. Fly, Z. Wang and J. E. Klaunig. *Indiana University, Bloomington, IN.*

Performance of working memory during complex cognitive tasks is dependent on multiple factors including age and diet. Oxidative stress has been reported to reduce cognitive function. Consumption of green tea, a rich source of antioxidants, has been associated with lower risk of cognitive impairment in epidemiological studies. Similarly, short term tea treatment was found to increase activity in the dorsolateral prefrontal cortex, an area that is critical in working memory processing. The aim of this study was to examine the effect of green tea extract on working memory in healthy younger (18–30 y) and older (50–70 y) women using a single-blind, placebo-controlled, crossover design. Twenty non-smoking Caucasian women were recruited and placed in either the younger (10) or the older (10) age group (IU IRB protocol #1406408282). Subjects received either 6 doses of green tea extract (totaling 5400 mg, with 45% epigallocatechin-3-gallate) or placebo (cornstarch) over a 24-hour period. Working memory capacity was measured by a battery of tests (Inquisit 4.0, Millisecond Software), including N-back task paradigm, reading span, and operation span. A 24-hour dietary recall was conducted for each treatment period to ensure similar dietary patterns. Differences were analyzed using a paired t-test. Green tea extract significantly improved reading span performance in 20 subjects, indicated by higher scores of recalled span sets and total letters compared to placebo. The number of total recalled letters increased in the 50-70 y group but not the 18-30 y group. Operation span was not different after green tea consumption in either group of subjects. The accuracy in 1-, 2-, and 3-back tasks was not significantly different across treatment. The reaction time of older women in the 1-back task was significantly shorter compared to placebo. Measurement of markers for oxidative stress in blood showed a reduction by tea consumption. In conclusion, acute consumption of green tea extract improved some but not all markers of working memory.

1457 A Comprehensive Toxicological Safety Assessment of an Extract of *Olea europaea* L. Leaves (Bonolive®)

A. Clewell[1], E. Béres[2], A. Vértesi[2], R. Glávits[2], G. Hirka[2], J. Endres[1], T. Murbach[1] and I. Pasics[2]. [1]*AIBMR Life Sciences, Inc., Puyallup, WA and* [2]*Toxi-Coop Zrt., Budapest, Hungary.*

Bonolive® is a commercial (Ferrer HealthTech) water-soluble extract prepared by hydroalcoholic extraction of the leaves of the olive tree, *Olea europaea* L. The extract is standardized to 40% oleuropein, and has been studied with regard to its effects on bone and cardiovascular health promotion. While available evidence suggests that olive leaves and olive leaf extracts have a relatively good safety profile, there have been a few inconsistencies in the literature, and the specific composition of an extract may influence both its efficacy and safety. Therefore, a battery of toxicological studies was conducted in accordance with internationally accepted standards and protocols to investigate the *in vitro* and *in vivo* genotoxicity and repeated oral toxicity of Bonolive®. No evidence of mutagenicity was observed at concentrations up to 5000 µg/plate in a bacterial reverse mutation test (AMES) and up to cytotoxic concentrations in an *in vitro* mammalian chromosomal aberration test. There was also no genotoxic activity observed in an *in vivo* mouse micronucleus test at concentrations up to the limit dose of 2000 mg/kg bw. A 90-day repeated oral toxicity study was conducted in Crl:(WI)BR Wistar rats at doses of 0, 360, 600, and 1000 mg/kg bw/d. No treatment-related mortality or adverse effects were observed and no target organs were identified. Thus the NOAEL from the 90-day study was determined to be 1000 mg/kg bw/d for both male and female rats, the highest dose tested.

1458 A Toxicological Safety Assessment of an Extract of *Morus alba* L. Leaves

T. Marx[1], R. Glávits[2], G. Hirka[2], J. Endres[1], A. Clewell[1] and I. Pasics[2]. [1]*AIBMR Life Sciences, Inc., Puyallup, WA and* [2]*Toxi-Coop Zrt., Budapest, Hungary.*

Morus alba (white mulberry) leaves have been consumed for millennia as a traditional Chinese medicine, and in a few Asian countries the leaves and leaf juice are considered traditional food and drink. Reducose (Phynova Group Limited) is a commercial water-soluble extract of *M. alba* leaves standardized to 5% 1-deoxynojirimycin (DNJ; an iminosugar with alpha-glucosidase inhibition properties). Available evidence suggests that M. alba leaves and extracts have a relatively good safety profile, however we are unaware of any safety assessments on an extract containing 5% DNJ or higher. The current study is a 28-day repeated dose study using Reducose in Wistar rats, performed according to OECD Guideline No. 407. Four groups of 10 animals per sex per group were given doses of 0, 1000, 2000 and 4000 mg/kg bw/day by gavage. No treatment-related mortality or adverse effects (per clinical observations, body weight/weight gains, food consumption, ophthalmoscopy, clinical pathology, gross pathology, organ weights or histopathology) were observed and no target organs were identified. Thus the NOAEL was determined to be 4000 mg/kg bw/d for both male and female rats, the highest doses tested.

1459 A Comprehensive Toxicological Safety Assessment of the Purine Alkaloid Theacrine

J. R. Endres[1], N. Deshmukh[2], R. Glávits[3], G. Hirka[3], A. Clewell[1], T. Marx[1] and I. Pasics[3]. [1]*AIBMR Life Sciences, Inc., Puyallup, WA;* [2]*INTOX Pvt. Ltd., Pune, India and* [3]*Toxi-Coop Zrt., Budapest, Hungary.*

Theacrine is a methylurate purine alkaloid, similar in structure to caffeine, which is a related methylxanthine alkaloid. It is found naturally in *Theobroma grandiflorum*, various *Coffea* species, and *Camellia assamica* var. kucha. Little information has been published on the safety/toxicity of theacrine. In the current study, a battery of toxicological studies was conducted in accordance with internationally accepted protocols to investigate the *in vivo* genotoxicity and repeated dose oral toxicity of Teacrine®, a commercial theacrine product standardized to >98% purity. No genotoxicity of Teacrine® was found in an *in vivo* mouse micronucleus study at concentrations up to 325 mg/kg bw per day. In a 14-day repeated dose study in Wistar rats, dosing at 500 mg/kg resulted in deaths of 5/5 males and 3/5 females with signs of tremors in all animals, while dosing at 400 mg/kg w/day caused death of one male rat. The NOAEL for the 14-day study was 350 mg/kg bw/day. A 90-day repeated oral toxicity study was performed in Wistar rats using gavage doses of 180, 300 and 375 mg/kg bw/day. Two females died in the 300 mg/kg bw/day group (days 33 and 67) and one male died in the 375 mg/kg bw/day group (day 42). Rats treated daily at 375 mg/kg bw/day revealed a slight reduction in body weight gain, food consumption and feed efficiency, and lowered weights of testes and epididymides. The latter correlated histologically to a decreased intensity of sperm formation and maturation. Above effects on testes and epididymides were also evident in males treated at 300 mg/kg bw/day, and were not accompanied by inflammatory or degenerative changes. There were no toxicologically relevant findings encountered in rats from the low dose group, making the NOAEL for Teacrine® 180 mg/kg bw/day in male and female Wistar rats in the 90-day study.

1460 Regulatory Effects of Platycodin D on Bone Remodeling

H. G. Jeong[1], J. H. Choi[1], Y. Han[2], H. G. Kim[1], H. M. Jeong[2], H. S. Lee[4], Y. C. Chung[3] and K. Y. Lee[2]. [1]*College of Pharmacy, Chungnam National University, Daejeon, Korea, Republic of;* [2]*College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju, Korea, Republic of;* [3]*Department of Food Science, International University of Korea, Jinju, Korea, Republic of and* [4]*Natural Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, Republic of.*

Postmenopausal osteoporosis is known to result from an imbalance between bone formation and resorption, leading to altered bone microarchitecture in women. In this study, we examined the inhibitory effects of saponins derived from *Platycodon grandiflorum* root (Changkil saponins: CKS) on osteoporosis induced by ovariectomy (OVX) in mice. Four weeks after surgery, the OVX mice were randomly divided into three groups and orally treated with saline or CKS for 3 weeks. Administration of CKS significantly reduced OVX-induced bone loss and ameliorated the reductions in the plasma levels of alkaline phosphatase, calcium, and phosphorus. CKS also prevented the deterioration of bone trabecular microarchitecture in OVX mice. Interestingly, platycodin D, the most abundant and major pharmacological constituent of triterpenoid CKS, increased alkaline phosphatase activity and enhanced mineralization process in osteobalst cells via the sirtuin/β-catenin pathways. Moreover, platycodin D inhibited RANKL-induced activation of NF-κB, and of the ERK and p38 MAP kinases, and ultimately repressed osteoclast differentiation. Taken together, these results suggest that the platycodin D could be an effective therapeutic compound for osteoporosis due to its dual regulatory effects on both osteoblast and osteoclast.

1461 The Effect of Administering Multiple Doses of Tall Larkspur (*Delphinium barbeyi*) to Cattle

K. D. Welch, B. T. Green, D. R. Gardner, D. Cook and J. A. Pfister. *USDA/ARS/PPRL, Logan, UT.*

Larkspurs (*Delphinium* spp.) are one of the most serious toxic plant problems on foothill and mountain rangelands in the western US. A considerable amount of research has been conducted over the years in both field and pen settings. The results of these research efforts have significantly increased our understanding of the poisoning of cattle by larkspurs. However, most of the pen studies conducted thus far have utilized a dosing regimen of a single bolus dose, which does not accurately mimic the manner by which cattle are poisoned by larkspur while grazing. Consequently, the objective of this study was to evaluate the acute toxicity of tall larkspur when administered in multiple doses, with the intent to identify a No Observable Adverse Effect Level (NOAEL). Hereford steers were administered various doses of tall larkspur at 12 h intervals for 4 d or until they showed marked signs of muscle weakness. The results suggest that a dose of 2 mg/kg/d N- (methylsuccinimido) anthranoyllycoctonine (MSAL)-type alkaloids is the NOAEL for a tall larkspur population with a norditerpenoid alkaloid profile containing 4 mg MSAL-type/g plant material and 12 mg non MSAL-type alkaloids/g plant material. Additionally, a computer model was generated to simulate multiple-dosing regimens at the various doses as well as different dosing regimens. The results from this study suggest that a 500 kg steer can consume a daily dose of 1.25 kg of fresh tall larkspur (with a similar alkaloid profile) without becoming severely poisoned.

1462 Toxicological Evaluation of *Striga hermonthica* Leaves Extract in Wistar Albino Rats

M. M. Onakpa, P. Adamu and D. Akumka. *Department of Veterinary Pharmacology and Toxicology, University of Abuja, Abuja, Nigeria.*

Striga hermonthica is a traditional herbal medicine used in Nigeria and west Africa; it is commonly called "witch weed" and locally known as "wuta wuta" among the Hausas of Northern and central Nigeria. This ubiquitous and hemi-parasitic plant growing in wide spectrum around food crops in Nigeria is widely used by traditional herbalists in humans to treat dermatosis, leprosy, ulcer, pneumonia, and to manage jaundice. It is also used used as an abortifacient, trypanocidal and as a purgative in treating bloat in ruminants. The objectives of this study were to investigate the acute and sub-acute toxicity effects of *Striga hermonthica* leaf extract in Wistar albino rats. In the acute toxicity test, a limit dose of 2000 mg/kg 80% methanolic leaf extract *S. hermonthica* was administered orally to the rats and then observed individually for 72 hours post dosing, and at least once daily for 28 days. Sub-acute toxicity was evaluated after administration of daily oral doses of 25 mg/kg, 50 mg/kg and 100 mg/kg body weight for 28 days. Hematology, serum biochemistry, histopathology as well as body and relative organ weights of the rats were evaluated. The limit dose of 2000 mg/kg did not cause any mortality or signs of toxicity in the rats tested during the observation period. In the sub-acute test, the results did not show any treatment related abnormalities in terms of hematological and biochemical parameters. The weekly body and organ weight of the rats showed no significant differences ($p > 0.05$) between the control and the rats treated with the extract. Results of this study suggest that 80% methanolic leaf extract of *Striga hermonthica* is relatively safe in rats when administered orally.

1463 Oral Administration of *Archachatina marginata* Hemolymph Prevents Renal Damage in Adrenaline-Induced Hypertensive Wistar Rats

A. B. Ojekale[2], P. Jewo[1], U. Agbafor[2] and J. Oguntola[1]. [1]*Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria and* [2]*Department of Biochemistry, Lagos State University, Ojo, Lagos, Nigeria.*

The meat of *Archachatina marginata* is commonly consumed as a source of protein, while the heamolymph is consumed as a antihypertensive by the Yorubas of South west Nigeria. This study evaluated the effects of oral administration of the hemolymph of *Archachatina marginata* on kidney on liver and kidney of adrenaline induced hypertensive wistar rats. Groups of adrenaline induced hypertensive wistar rats were orally administered with 22.8 mg/kg body weight filtered hemolymph, and nifedipine (positive control) daily for seven days. The animals were sacrificed post peritoneal anesthesia with urethane: chlorase mixture. Blood was collected and analysed for marker enzymes and metabolites of the liver and Kidney. The kidney and liver samples were histologically evaluated. Kidney damage was prevented in the group administered with the hemolymph of *Archachatina marginata* compared with the distil water (negative control) treated group. Elevated levels of aspartate amino transferase, alanine amino transferase and alkaline phosphatase activities (liver function biomarkers) were observed in the 22.8 mg/kg body weight hemolymph treated rats. No statistically significant difference ($p<0.05$) were observed in the urea, globulin, albumin, bilirubin, creatinine, total protein levels among the different groups of animals used.

1464 *Gymnema sylvestre* Prevents Initial Progression of Obesity Induced by a High-Fat Diet in C57bl/6j Mice

H.-J. Kim and M.-H. Cho. *College of Veterinary Medicine, Seoul National University, Seoul, Korea, Republic of.*

The anti-obesity effect of *Gymnema sylvestre* (GS) has been widely reported; however, it has not yet been elucidated whether GS could prevent the initial progression of high-fat diet (HFD)-induced obesity in normal, nonobese mice. To investigate this issue, male C57BL/6J mice were fed on either a normal diet, HFD, or HFD containing 1% GS (HFD+GS) for 4 weeks. The HFD+GS group had a decreased body weight gain, serum levels of total cholesterol, triglyceride, low-density lipoprotein-cholesterol, and leptin compared with the HFD group. Moreover, retroperitoneal as well as epididymal fat weight and adipocyte hypertrophy, lipid droplets in liver, and serum levels of aspartate aminotransferase and alanine transaminase were also decreased. These results indicate that GS had an initial anti-obesity effect as well as a preventative effect for potential liver injury induced by a HFD. Keyword: *Gymnema sylvestre*, Obesity, Body weight gain, High-fat diet, Adipocyte. Acknowledgments: This work was supported by the Bio-Synergy Research Project (NRF-2012M3A9C4048819) of the Ministry of Science, ICT and Future Planning through the National Research Foundation.

1465 Abstract Withdrawn by Author.

1466 Toxicological and Reversibility Assessment of *Dalbergia saxatilis* Hook, F. (*Papilionaceae*) Root Extract in Rats

O. K. Yemitan[2] and O. O. Adeyemi[1]. [1]*Department of Pharmacology, Therapeutics and Toxicology, College of Medicine of the University of Lagos, Lagos, Nigeria and* [2]*Department of Pharmacology, Therapeutics and Toxicology, Lagos State University College of Medicine, Lagos, Nigeria.* Sponsor: P. Wright.

The aqueous root extract of *Dalbergia saxatilis* (*DS*), is popular in Africa, for chronic conditions, as anticonvulsant and neurosedative and analgesic. *DS* was investigated for its effects on various toxicological endpoints in rats, with a view to ascertaining and predicting its safety claims in humans. Rats of both sexes were given daily doses of *DS* (40, 200 or 1000 mg/kg) for 90 days, during which the body weights were measured weekly. After 90 days, weights of vital organs were measured; serum metabolites- alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), bilirubin and uric acid were measured; and oxidative stress markers- catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) were determined from liver and spleen tissues. Hematologic and spermatologic functions were also assessed. These were followed by 14 days reversibility tests. There was significant (P = .05) body weight gain per week at 40 mg/kg & 200 mg/kg *DS* groups. Significant increases were also recorded in liver weight of female rats at 1 g/kg and spleen at 200 mg/kg for female & 1 g/kg for rats of both sexes. ALP was significantly elevated in female rats at 200 mg/kg, and in both sexes at 1/g/kg; direct bilirubin was elevated at 1 g/kg in both sexes; whereas LDH was elevated in female rats at 1 g/kg. Significant elevation of CAT, SOD & GSH, and lowering of MDA were recorded for female rats at 1 g/kg. There was significant irreversible reduction of leucocytes and spermatozoa counts. Histopathology of hepatocytes revealed lesions at 1g/kg *DS*. In conclusion, *DS* could potentially cause various irreversible toxicities on the white blood cells, spermatozoa and liver during prolonged oral use at high doses; contradicting its safety claims.

1467 Chemomodulatory Action of 4-methylthio-3-butenyl Isothiocyanate on Carcinogenesis in Rats

I. Suzuki[3,5], Y.-M. Cho[3], T. Hirata[2,3], T. Toyoda[3], J.-i. Akagi[3], A. Nishikawa[3], Y. Nakamura[4] and K. Ogawa[3]. [1]*Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan;* [2]*Department of Pharmacology, Showa University, Tokyo, Japan;* [3]*Division of Pathology, National Institute of Health Sciences, Tokyo, Japan;* [4]*Faculty of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan and* [5]*Pathogenetic Veterinary Science, Gifu University, Gifu, Japan.*

Isothiocyanates (ITCs) showed chemopreventive effects in several kinds of animal tumor models at low doses. On the other hand, some ITCs at high doses are known to cause toxicity and enhance carcinogenesis in rat urinary bladder. As for 4-methylthio-3-butenyl isothiocyanate (MTBITC), an ingredient of Japanese white radish, there is limited information for chemopreventive effect, and *in vivo* toxicity has not yet been investigated. In this study, we examined toxicity and modifying effects of MTBITC on carcinogenesis in rats. To evaluate inhibitory effects of MTBITC on rat esophageal carcinogenesis, rats were subcutaneously injected with N-nitrosomethylbenzylamine (NMBA) for initiation, and fed an 80 ppm MTBITC-diet in the initiation stage and/or promotion stage. The multiplicity of neoplastic lesions in esophagus was significantly decreased in MTBITC-treated groups as compared with the NMBA-alone group. Immunohistochemical analysis revealed that MTBITC induced apoptosis and suppressed the cell proliferation when treated in the promotion phase. To elucidate toxicity of MTBITC in the urinary bladder in rats, rats were fed with up to 1000 ppm MTBITC-diet for 14 days. Significant increase of organ weight, and mild histopathological degenerative changes in the urinary bladder were seen at 1000 ppm of MTBITC but not 300 ppm or less. Then, comet assay in the urinary bladder and micronucleus assay in the bone marrow revealed % tail DNA and micronucleated polychromatic erythrocytes were not affected by MTBITC treatment. These results suggest toxicity of high dose-MTBITC in rat urinary bladder is induced through non-genotoxic mechanism. In conclusion, it is important to clarify the borderline level of beneficial effect and toxicity in MTBITC.

1468 The Inhibitory Effects of *Salvia plebeia* on Atopic Dermatitis-Like Skin Lesions

J. K. Choi[2], S. Lee[3] and S.-H. Kim[2]. [1]*Department of Pharmacology, Kyungpook National University, Daegu, Korea, Republic of;* [2]*Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu, Korea, Republic of and* [3]*Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, Korea, Republic of.*

Salvia plebeia R. Br. (Lamiaceae) has been used for folk medicines in Asian countries, including Korea and China, to treat skin inflammatory diseases and asthma. In this study, we investigated the effects of *S. plebeia* extract (SPE) on atopic dermatitis (AD)-like skin lesions and defined underlying mechanisms of action. We established an AD model in BALB/c mice by repeated local exposure of house dust mite extract (*Dermatophagoides farinae* extract, DFE) and 2,4-dinitrochlorobenzene (DNCB) to the ears. Repeated alternative treatment of DFE/DNCB caused AD-like skin lesions. The oral administration of SPE decreased AD symptoms based on ear thickness and histopathological analysis, in addition to serum IgE and IgG2a levels. SPE suppressed mast cell infiltration into the ear and serum histamine level. SPE inhibited Th1/Th2/Th17 phenotype CD4(+) T lymphocytes expansion in the lymph node and the expression of Th1/Th2/Th17 cytokines in the ear tissue. To define the underlying mechanisms of action, the tumor necrosis factor (TNF)-α and interferon (IFN)-γ activated human keratinocytes (HaCaT) model was used. SPE significantly suppressed the expression of cytokines and chemokines through the down-regulation of mitogen-activated protein kinases, nuclear factor-κB, and STAT1 in HaCaT cells. Taken together, our results suggest that SPE might be a candidate for the treatment of AD.

1469 Inhibitory Effects of Capsaicin on Dimethylnitrosamine-Induced Hepatic Fibrosis in Rat

J. H. Choi[1], Y. C. Chung[2] and H. G. Jeong[1]. [1]*College of Pharmacy, Chungnam National University, Daejeon, Korea, Republic of and* [2]*College of Public Health and Natural Science, International University of Korea, Jinju, Korea, Republic of.*

Liver fibrosis is a wound healing response to a variety of chronic stimuli, including alcohol intake, viral infection, drugs, and metabolic disease. During the development of liver fibrogenesis, quiescent hepatic stellate cells proliferate and undergo a phenotypic transformation to myofibroblast-like cells. This study investigated the protective effects of capsaicin on dimethylnitrosamine (DMN)-induced liver fibrosis in rats. DMN treatment for 4 weeks led to marked liver fibrosis as assessed by serum biochemistry, histopathological examination, and hepatic lipid peroxidation and collagen content. Capsaicin treatment significantly inhibited DMN-induced increases in serum ALT and AST activities, fibrosis score, and hepatic malondialdehyde and collagen content. Capsaicin also inhibited DMN-induced reductions in rat body and liver weights. RT-PCR revealed that capsaicin inhibited DMN-induced increases in MMP-13, TIMP-1, and TNF-α mRNA. In addition, the protein expression levels of transforming growth factor-βI(TGF-βI), smad2 , smad3, collagen type I and α-smooth muscle were downregulated specifically by capsaicin treatment, whereas the protein expression levels of smad7 and PPARγ were upregulated. Taken together, these findings suggest that capsaicin regulates TGF-βI, PPARγ and SMAD-dependent mechanism in blocking fibrotic development and provide a promising strategy for hepatic fibrosis treatment.

1470 Inhibitory Effects of Saponins from the Roots of *Platycodon grandiflorum* on High-Fat Diet-Induced Non-Alcoholic Steatohepatitis by Inducing Antioxidant Enzymes and Fatty Acid Oxidation

Y. C. Chung[3], J. H. Choi[2], C. Y. Choi[1], S. W. Jin[2], H. G. Kim[2], S. J. Kim[2], Y. C. Lee[4] and H. G. Jeong[2]. [1]*Jeollanamdo Institute of Natural Resources Research, Jangheung, Korea, Republic of;* [2]*College of Pharmacy, Chungnam National University, Daejeon, Korea, Republic of;* [3]*College of Public Health and Natural Science, International University of Korea, Jinju, Korea, Republic of and* [4]*Jangsaeng Doraji Research Institute of Biotechnology, Jangsaeng Doraji Co., Ltd., Jinju, Korea, Republic of.*

Platycodon grandiflorum has been healthy effects due to its various nutritious compounds and is considered as a functional food. Several studies have indicated that *Platycodon grandiflorum* root-derived saponins (Changkil saponins: CKS) exhibits anti-oxidative and anti-inflammatory, but the effect of CKS on non-alcoholic steatohepatitis (NASH) remains unclear. This study evaluate the inhibitory effects of CKS on high fat diet (HFD) induced NASH. CKS administration suppressed HFD-induced hepatic malondialdehyde level, collagen deposition, mRNA expression of TIMP-1, TNF-α, IL-1β and IL-6, and protein expression of TGF-β1, α-SMA, and type I collagen. CKS treatment inhibited HFD-induced COX-2 expression via inhibition of NF-κB p65 nuclear translocation and IκBα degradation. CKS treatment restored HFD-reduced Nrf2-mediated HO-1, NQO1, and GST expression. Furthermore, CKS treatment reinstated HFD-reduced PPARα-regulated ACOX and CPT-1 expression. These results indicated that CKS ameliorates HFD-induced hepatic oxidative stress, inflammation, and fibrosis via induction of Nrf2-mediated anti-oxidant enzymes and PPARα-regulated fatty acid oxidation.

1471 Toxic Effects of the Aqueous Root Extract of *Talinum triangulare* As Investigated in Rodents

F. R. Aigbe, M. O. Oyatorisa and O. O. Adeyemi. *Pharmacology, Therapeutics and Toxicology, University of Lagos, Lagos, Nigeria.*

Talinum triangulare, commonly called "water leaf" is a cosmopolitan weed in humid tropics that is eaten as leafy vegetable with medicinal properties in most of such regions. The aqueous extract of its root is also used to treat various ailments including asthma and hypertension. This study was aimed at investigating the toxic potential of the aqueous root extract of *Talinum triangulare* (ARETT). Acute (24 hours) and sub chronic (90 days) toxicity studies on the extract were carried out in mice and rats respectively. The oral and intraperitoneal acute toxicity tests revealed LD50 of 5623 mg/kg and 398 mg/kg respectively in mice. In the sub chronic toxicity test, ARETT significantly reduced body weight of rats. It also brought about significant reduction in the leukocytes, erythrocytes, haemoglobin and platelet counts; elevation of serum cholesterol, uric acid and creatinine, while reducing total protein of exposed rats. Serum oxidative stress markers' assay revealed significant ($p<0.001$) reductions in SOD, catalase, glutathione and glutathione peroxidase levels and an elevation of MDA concentration in the liver homogenates of exposed rats. Histological examinations also indicted that interstitial inflammation occurred in kidney tissues of treated rats. The findings of this study show that the aqueous root extract of *Talinum triangulare* is toxic; inducing weight loss, anaemia, immunosuppression and oxidative tissue damage. Extreme caution should therefore be exercised in its therapeutic applications, especially on a long term basis.

1472 Effect of Rutaecarpine in Tert-butyl Hydroperoxide-Induced Apoptosis by Up-Regulating Phase II Enzymes through TRPV1/CaMKII/Akt Pathway in HepG2

S. S. Won[1], S. W. Jin[1], Y. P. Hwang[2] and H. G. Jeong[1]. [1]*College of Pharmacy, Chungnam National University, Daejeon, Korea, Republic of and* [2]*Department of Pharmaceutical Engineering, International University of Korea, Jinju, Korea, Republic of.*

Rutaecarpine, a major quinazolinocarboline alkaloid from evodia rutaecarpa, was reported to show pharmacological activities, such as vasodilation, anti-thrombosis, and anti-inflammation. However, the anti-oxidative effect of rutaecarpine and its underlying pathway remain unclear. This study investigated the protective effects of rutaecarpine on tert-butyl hydroperoxide (t-BHP)-induced oxidative stress leading to apoptosis in HepG2. Pretreatment of rutaecarpine significantly reduced t-BHP-induced apoptosis and ROS generation. Rutaecarpine up-regulated phase II enzymes, including HO-1, NQO1 and GCSc expression and, nuclear translocation of Nrf2. Also, rutaecarpine increased the phosphorylation of Akt and MAPKs but pretreatment with Akt inhibitor (LY294002) only significantly inhibited rutaecarpine induced-HO-1 expression. Furthermore, TRPV1 receptor (SB366791) and CaMKII (KN-93) inhibitiors suppressed rutaecarpine induced-HO-1 expression and Akt phosphorylation. These results indicate that rutaecarpine could be beneficial in hepatic diseases by up-regulating the phase II enzymes via TRPV1/CaMKII/Akt signaling pathways.

1473 Effect of Betulinic Acid on eNOS Phosphorylation and No Synthesis via Calcium Signaling Pathway

S. W. Jin, S. J. Kim and H. G. Jeong. *College of Pharmacy, Chungnam National University, Daejeon, Korea, Republic of.*

Endothelial nitric oxide synthase (eNOS) is the key regulatory enzyme in the vascular endothelium. Betulinic acid was reported to attenuate vascular diseases and atherosclerosis, but its eNOS stimulating mechanism is not elucidated. This study examined the intracellular pathways underlying eNOS activity and endothelial nitric oxide (NO) production by betulinic acid in EA.hy926 cells. Betulinic acid treatment induced the phosphorylation of eNOS on Ser1177 and NO production. Betulinic acid increased intracellular calcium level and phosphorylation of calcium/calmodulin (CaM) dependent kinases, CaMKIIα and CaMKKβ. L-type calcium channel (LTCC) and ryanodine receptor (RyR) inhibition abolished betulinic acid-induced intracellular calcium levels and eNOS phosphorylation. Treatment with W7, a CaM antagonist, KN-93, a selective inhibitor of CaMKII and STO 609, a selective inhibitor of CaMKK, suppressed on eNOS phosphorylation and NO production. Moreover, AMPK was induced by betulinic acid and betulinic acid-induced eNOS phosphorylation was inhibited by compound C, AMPK inhibitor. Taken together, these results indicate that betulinic acid activates eNOS phosphorylation and NO synthesis via calcium/CaMKII and via calcium/CaMKK/AMPK pathways. These findings provide further insight into the eNOS signal pathways involved in the anti-atherosclerosis effects of betulinic acid.

1474 Some Natural Amino Acids Ameliorate Metabolic Dysfunction by Activating SIRT1

W.-C. Sim, Y.-J. Choi, D. G. Kim and B.-H. Lee. *Seoul National University, Seoul, Korea, Republic of.*

SIRT1, a NAD-dependent deacetylase, plays major roles in regulation of cellular metabolism. SIRT1 is known to increase mitochondrial biogenesis and function by deacetylating PGC-1a, a master regulator of mitochondrial biogenesis. We evaluated the effects of amino acids on SIRT1 activity and found some SIRT1-activating amino acids (SAAs). In C2C12, a mouse myoblast cell, SAAs increased intracellular NAD+ level, which is a substrate of SIRT1. By increasing NAD+, the amino acids deacetylated PGC-1α and mitochondrial gene expression. The SAAs also upregulated mitochondrial function and inhibition of SIRT1 by si-SIRT1 or EX-527, a inhibitor of SIRT1, reversed mitochondrial gene expression

and function to control level. The SAAs decreased lipid accumulation by upregulating gene expression associated with fatty acid β-oxidation and improved insulin resistance. The SAAs reversed palmitate-induced Akt-dephosphorylation and upregulated translocation of GLUT4 to the membrane and uptake of glucose. These results indicate that The SAAs have the potential of therapeutic for insulin resistance and obesity by increasing SIRT1 activity.

1475 Microcystin-LR Promotes Liver Cell Proliferation by Activating Akt/mTORC1/S6K1 Pathway

J. Liu, B. Wang and L. Xu. *School of Medicine, Zhejiang University, Hangzhou, China.* Sponsor: H. Wang.

Microcystin-LR (MC-LR), heptapeptides produced by blue-green algae, was shown to inhibit protein phosphatase 2A (PP2A) activity and hyperphosphorylated PP2A substrates at 24 h exposure in human normal liver cell line HL7702 in our previous studies, but no other cellular effects was observed. In this study, prolonged MC-LR exposure was designed to further discover its adverse effects. HL7702 cells were exposed to 1-10 μM MC-LR for 1-72 h and ICR mice were injected intraperitoneally with 20-80 μg/kg MC-LR for 2 h-4 d. The results show that the uptakes of MC-LR and its binding with PP2A/C were concentration-dependent but time-independent, while the inhibition of total PP2A activity was both concentration and time dependent in HL7702 and mice liver cells. MC-LR did not further reduce PP2A activity after 24 h exposure in HL7702 cells. The PP2A regulator α4 was found releasing its associated PP2A/C in both HL7702 cells and mice liver since MC-LR binding to PP2A/C. It was found that MC-LR promoted HL7702 cells proliferation after 36 h exposure and the mice liver cell proliferation after 1 d injection. p-Akt-T308, p-Akt-S473, p-S6K1, p-S6, and p-4E-BP1 increased in a concentration dependent manner since 1 h exposure in HL7702 cells and 2 h exposure in mice liver, suggesting that Akt/mTORC1/S6K1 cascade was activated as early as MC-LR binding to PP2A/C. With rapamycin, we demonstrated that MC-LR-activated S6K1 was due to both the hyperphosphorylation of Akt and the inhibition of PP2A activity toward S6K1. Moreover, the PI3K/Akt inhibitor LY294002 blocked MC-LR-induced activation of Akt/mTORC1/S6K1 pathway and cell proliferation. Furthermore, the activations of ERK/p38/JNK were also observed in both HL7702 and mice liver cells after MC-LR exposure. Taken together, the present study demonstrates that MC-LR exposure enhanced cell proliferation and the activation of Akt/mTORC1/S6K1 pathway was the main mechanism. The inhibition of PP2A was the main reason for the molecular changes. Meanwhile, the auto-regulation mechanism of PP2A by α4 might be involved in the process.

1476 Effects of Chemopreventive Natural Compounds on Function and Expression of Human P-glycoprotein

T. Nabekura, T. Kawasaki, M. Furuta, T. Kaneko and Y. Uwai. *School of Pharmacy, Aichi Gakuin University, Nagoya, Japan.* Sponsor: M. Satoh.

Drug efflux transporter P-glycoprotein (MDR1, ABCB1) plays important roles in absorption, distribution and elimination of xenobiotics. The nuclear factor-kappaB (NF-κB) transcription factors play critical roles in development and progression of cancer. Several dietary natural compounds are known to block the NF-κB activation process, and thus act as cancer chemopreventive agents. In this study, we investigated the interaction of natural compound that is reported to inhibit NF-κB activation with human P-glycoprotein. Human MDR1 gene-transfected KB/MDR1 cells and fluorescent substrates of P-glycoprotein, daunorubicin and rhodamine 123, were used to evaluate the function of P-glycoprotein. The accumulation of daunorubicin or rhodamine 123 in KB/MDR1 cells increased in the presence of caffeic acid phenetyl ester (CAPE), anacardic acid, celastrol, xanthohumol, magnolol, and honokiol in a concentration-dependent manner. The ATPase activities of P-glycoprotein were stimulated by CAPE, anacardic acid, celastrol, xanthohumol, magnolol, and honokiol. KB/MDR1 cells were sensitized to vinblastine cytotoxicity by CAPE, anacardic acid, xanthohumol, magnolol, and honokiol, showing that these natural compounds reverse multidrug resistance. CAPE, anacardic acid, xanthohumol, magnolol, and honokiol induced transcriptional activity of MDR1 promoter in human intestinal epithelial LS174T cells. These results suggest that dietary chemopreventive natural compounds, such as CAPE, xanthohumol, magnolol, and honokiol, have inhibitory and inducible effects on P-glycoprotein and potencies to modulate toxicokinetics of xenobiotics.

1477 Epigallocatechin-3-Gallate Enhances Benzo[a] pyrene-Induced Cytotoxicity Through Increased Reactive Oxygen Species Production in Human Lung Epithelial Cells

M. Cromie, Q. Wang and W. Gao. *Environmental Toxicology, Texas Tech University, Lubbock, TX.* Sponsor: M. Cromie.

Our recent study found that curcumin and vitamin E protected against the cellular and molecular damage in human lung epithelial cells (BEAS-2B) upon exposure to the well-studied carcinogen, benzo[a] pyrene (BaP). Based on these findings, we further assessed whether epigallocatechin-3-gallate (EGCG), the primary catechin in green tea, would exhibit similar characteristics. In the present study, we used trypan blue assay, fluorescence microplate assay, flow cytometry, and qRT-PCR to measure cell viability, reactive oxygen species (ROS), cell cycle, and gene expression in BEAS-2B cells exposed to BaP (5 μM), EGCG (20 μM), or BaP+EGCG. Trypan blue assay revealed that BaP-induced cytotoxicity was significantly magnified by EGCG co-treatment ($P<0.01$). As expected, a significant increase in ROS was observed in BaP-exposed cells, and EGCG co-treatment significantly stimulated BaP-promoted ROS formation at 4 and 8 hours ($P<0.05$). Additionally, flow cytometry analysis revealed significant G2/M arrest in co-treated cells after 24 hours ($P<0.05$). qRT-PCR results demonstrated a >100-fold increase in cytochrome P450 1A1 (CYP1A1) upon BaP exposure, but this increase was reduced to approximately 70-fold in cells co-treated with EGCG+BaP ($P<0.01$). Although no significant changes in gene expressions of superoxide dismutase 1/2 (SOD1/2) and glutathione peroxidase 1 (GPX1) were observed, cells treated with EGCG or BaP+EGCG had a >5-fold decrease in catechol-O-methyltransferase (COMT) expression compared to the control ($P<0.05$). Our previous study found curcumin and vitamin E had protective effects, whereas EGCG was found to augment the cytotoxicity of BaP in EGCG co-treated cells. EGCG possibly achieved this through the inhibition of CYP1A1 and COMT, which are important enzymes involved in BaP metabolism. Overall, EGCG co-treatment could delay BaP metabolism and potentially contribute to a greater induction of ROS, and therefore result in an increased cytotoxic effect in BEAS-2B cells.

1478 Cytotoxicity Characterization of the Novel Polyarsenical Arsenicin A in Human Cell Lines

B. Moe[1], L. Chen[1], W. R. Cullen[2], X.-F. Li[1] and X. C. Le[1]. [1]*University of Alberta, Edmonton, AB, Canada and* [2]*University of British Columbia, Vancouver, BC, Canada.*

Isolated from the organic extract of a marine sponge, Arsenicin A is the first polyarsenical found in nature. While it has been shown to be a potent fungicide and bactericide in human pathogenic strains, the cytotoxicity of this novel arsenical in human cell lines has not been demonstrated. Because some arsenic species (arsenic trioxide, As2O3) are actively used as antineoplastic drugs, the determination of the cytotoxic abilities of Arsenicin A in human cells will elucidate its potential use in cancer therapy as well. Arsenicin A (AA) and As2O3 (AsIII) were solubilized in water, sterile filtered, and calibrated using inductively-coupled plasma mass spectrometry (ICP-MS). Cytotoxicity was determined using real-time cell analysis (RTCA) in human carcinoma cell lines associated with target organs of arsenic-induced toxicity: A549 (lung), HepG2 (liver), and T24 (bladder). Cytotoxicity response profiles (cellular response over time) and IC50 histograms (IC50 values over time) were determined over 72 h exposure. Intracellular uptake of arsenicals was examined in A549 cells via ICP-MS analysis of total arsenic in cell lysate after 24 h exposure to concentrations equal to 24 h IC50 values. 24 h IC50 values were determined for each arsenical in all cell lines (A549: 7.3 ± 0.43 μM AA, 74.2 ± 4.1 μM AsIII; T24: 3.6 ± 0.24 μM AA, 6.9 ± 0.52 μM AsIII; HepG2: 3.5 ± 0.40 μM AA, 91.9 ± 4.8 μM AsIII). AA was more cytotoxic than AsIII in all cell lines on the basis of 24 h IC50 values ($p<0.001$; two-way ANOVA with Bonferroni post-test). Furthermore, AA was a broader cytotoxicant, as it was highly cytotoxic to all cell lines in comparison to AsIII, which was significantly more cytotoxic in T24 cells than the other cell lines ($p<0.001$). Analysis of intracellular uptake revealed that the total number of As atoms cell-1 was nearly four times higher in AA-treated A549 cells, indicating an equal number of intracellular AA and AsIII molecules, as one molecule of AA contains four As atoms. However, as the cells were treated with a molar concentration of AA (3.6 μM) that was one-twentieth the molar concentration of AsIII (76.6 μM), this indicates that uptake and/or retention of AA is much more efficient than AsIII in A549 cells. The enhanced cytotoxicity and uptake/retention of AA indicates it may have potential use in cancer therapy; further investigation into the biological mechanisms of this novel arsenical is warranted.

1479 Comparative Study of Microcystin-LR on PP2A in Various Tumor Cell Lines

L. Xu[2], H. Wang[2], K. Xu[2], B. Wang[2], J. Liu[2] and H. Wang[1]. *[1]2Toxicology Program, Department of Environmental and Occupational Health Science, University of Washington, Seattle, WA and [2]School of Medicine, Zhejiang University, Hangzhou, China.*

Protein phosphatase 2A (PP2A) is distinctive in its key role in cell and has been recognized as a tumor suppressor protein. Microcystin-LR (MCLR) produced by blue-green algae is a potent tumor promoter and the major mechanism has been known for its inhibition on PP2A by covalent binding to S269 of the PP2A/C as some cancer therapeutic medicine does for anti-cancer. Our interest is how MCLR affects PP2A and what role PP2A plays in MCLR treated cancer cell lines. Human hepatoma SMMC7721,laryngeal epithelial cells Hep2 and lung adenocarcinoma cell line A549 were treated with 0.1~10 μmol/L MCLR for 0~48h. The results show that MCLR entered into the cells and conjugated with PP2A/C. PP2A activities were all inhibited and A549 was the most sensitive cell line. The phosphorylation of PP2A/C at Y307, which is negatively related with PP2A activities, was increased in three cell lines but methylation of PP2A/C at L309 also responsible for PP2A activity was only changed in Hep2. Interaction between PP2A/C and PP2A regulator α4, which indicates a compensate mechanism for PP2A activity, was decreased in Hep2 and A549 but increased in SMMC7721. The disorganization of cytoskeleton can be found in three cell lines but the involvement of cytoskeleton-associated proteins dephosphorylated by PP2A were different, both p-VASP and p-Hsp 27 were increased in SMMC7721 and A549 but only p-VASP in Hep2. The change of cell proliferation by MCLR for more than 24 h was only observed in A549 but not in the others. In summary, our study suggests that MCLR can bind with PP2A/C and inhibit its activity in three cell lines but the underling mechanisms and subsequent affected PP2A substrates and cellular outcome were different. The study will be meaningful in further elucidating the mechanisms of MCLR affecting PP2A as well as the regulatory role of PP2A in tumor cell. Meanwhile, the information may be helpful in understanding the mechanisms of cancer drugs that act on PP2A.

1480 Exposure of the Human HepaRG Liver Cell Line to Pyrrolizidine Alkaloids Results in a Gene Expression Profile Characteristic for Genotoxic Carcinogens

A. A. Peijnenburg, G. Stoopen, C. M. Polman, T. F. Bovee and P. J. Hendriksen. *RIKILT, Wageningen University and Research Centre, Wageningen, Netherlands.* Sponsor: J. Louisse.

Pyrrolizidine alkaloids (PAs) are secondary metabolites found in many plant species and can be present as contaminants in food, herbal teas, plant food supplements, and animal feed. A large number of PAs are toxic not only to livestock and wildlife but also to humans. Hepatotoxicity, genotoxicity, and carcinogenicity are the main toxicities observed in experimental animals treated with PAs. However, there is little direct evidence for mutagenicity and carcinogenicity of PAs in humans and epidemiological data are lacking. The present study aimed to contribute to a better evaluation of the genotoxic and carcinogenic risks of PAs for humans. To that end, the human hepatoma cell line HepaRG was exposed *in vitro* for 72 hours to six PAs (riddelliine, retrorsine, echimidine, monocrotaline, lasiocarpine, senkirkine), and the genotoxic carcinogen benzo[a]pyrene, the non-genotoxic carcinogen bis(2-ethylhexyl) phthalate, and the non-carcinogen mannitol as controls. Upon exposure, RNA was isolated and effects on whole genome mRNA expression were analysed using DNA microarrays. Pathway analysis showed that several processes involved in genotoxicity, including DNA damage response, p53 pathway and cell cycle checkpoints, were modulated by the PAs. Comparison of the array data with transcriptome data obtained by others upon exposure of HepaRG to various model hepatocarcinogens showed that each of the PAs used in the present study generated a gene expression profile that is characteristic for genotoxic carcinogens.

1481 Punicic, Jacaric, and Lithocholic Acid Induce ER Stress, Mitochondrial Dysfunction, and Autophagy in Human Prostate Cancer Cells

A. Gafar[1,3], H. Draz[1], J. Gasmi[1], A. Goldberg[1], M. Bashandy[3], V. Titorenko[2] and T. Sanderson[1]. *[1]INRS-Institut Armand-Frappier, Laval, QC, Canada; [2]Department of Biology, Concordia University, Montreal, QC, Canada and [3]Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.*

Punicic (PA) and jacaric (JA) acid are naturally-occurring fatty acids found in pomegranate and Jacaranda seed; lithocholic acid (LCA) is a secondary bile acid that undergoes enterohepatic circulation. Each of these compounds is selectively toxic to various human cancer cells and we have previously shown these compounds to induce apoptotic and necrotic death of prostate cancer cells, but not of normal prostate epithelial cells. Our objectives were to determine the involvement of ER stress, mitochondrial dysfunction and autophagy in the death triggered by these compounds in prostate cancer cells with various tumorigenic characteristics. PA reduced androgen-dependent LNCaP and -independent PC-3 cell viability with similar potency (IC50 18-27 μM), whereas JA was more toxic to these cells (IC50 8-10 μM). LCA killed PC-3 and DU-145 cells with IC50 values of 30-32 μM. All three compounds induced ER stress markers CHOP and phosphorylated eIF2α at relatively toxic concentrations (30-50 μM) after 24 h. The ER stress inhibitor salubrinal slightly reduced the cytotoxicity of PA, but exacerbated that of JA in the 1-10 μM range in LNCaP and PC-3 cells, with no effect at more toxic fatty acid concentrations. The toxicity of LCA was slightly enhanced by salubrinal in PC-3 and DU-145 cells. Inhibition of CHOP by siRNA had no effect on the toxicity of any of the compounds, regardless of cell type. PA and JA increased levels of reactive oxygen species in LNCaP and PC-3 cells within 1 h, as well as loss of mitochondrial outer-membrane potential (> 30 μM) and pretreatment of cells with the antioxidant tocotrienol protected against fatty acid-mediated cytotoxicity. We suggest that ER stress is a response to, but not essential for PA- and JA-mediated cell death, which appears to be triggered by ROS-induced mitochondrial dysfunction. LCA increased autophagy markers (GFP-LC3B punctuation and conversion of LC3BI to LC3BII) in PC-3 cells (but not in autophagy-deficient DU-145 cells). The autophagy inhibitor bafilomycin A1 increased the toxicity of subtoxic concentrations of LCA (3-10 μM), suggesting the autophagic response of PC-3 cells to LCA is protective in nature.

1482 Strategies to Protect Against Triptolide Based on Its Toxic Mechanism

W. Wang and Z. Huang. *School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China.* Sponsor: H. Raabe.

Triptolide is a major active ingredient of the Chinese herb *Tripterygium wilfordii* Hook f. (TWHF) and has risen to wide acceptance on its remarkable performance in recent scientific investigations, showing multiple biological activities, such as anti-inflammatory, anti-fertility, antineoplastic and immunosuppressive activities. However, the clinical applications of triptolide are limited by its narrow therapeutic window and severe toxicity, particularly nephrotoxicity, hepatotoxicity and cardiotoxicity. In previous study, we reported that apoptosis induced by triptolide was mediated by oxidative stress along with the damage of NF-E2-related factor 2 (Nrf2) system, which is the main antioxidant defense system. Meanwhile, autophagy, known as a cellular self-digestion process, is also involved in the effect of triptolide. Interestingly, Both Nrf2 system and autophagy are activated in a short time, while suppressed in a longer period of time. As basic defense mechanism, Nrf2 pathway and autophagy are adaptively activated in response to oxidative stress in a short time, while are overwhelmed by increasing stress that trigger the toxicity subsequently. Our lab focus on the role of Nrf2 and autophagy in triptolide-induced toxicity. Fortunately, activation of Nrf2 plays a protective role against triptolide-induced cytotoxicity in BALB/C mice, NRK-52E cells, HepG2 cells and H9c2 cells. Additionally, our recent study suggests that autophagy can selectively eliminate abnormal mitochondria damaged by reactive oxygen species (ROS). The induction of autophagy by rapamycin could ameliorate the detrimental effects induced by triptolide in BALB/C mice. Collectively, Nrf2 and autophagy may be the potential target for modulating toxicity induced by triptolide. More specific and low toxic agonists targeting Nrf2 and autophagy need to be further studied before the clinical application against triptolide-induced toxicity.

1483 Comparing Early Life Stage Effects in Zebrafish of Extraction Methods for *Stellera chamaejasme*, an Herbal Plant Used in Mongolian Medicine

W. Dong[2], J. Zong[2], M. Li[2], J. Yang[2], S. Tai[2], L. Xu[2], M. Chernick[3], D. E. Hinton[3], B. Yang[1] and W. Dong[2,3]. [1]*Department of Dermatology, People's Hospital of Hubei Province, Wuhan, China;* [2]*Inner Mongolia Provincial Key Laboratory for Toxicants and Animal Disease/Inner Mongolia University for the Nationalities, Tongliao, China and* [3]*Nicholas School of the Environment, Duke University, Durham, NC.* Sponsor: W. Dong.

Stellera chamaejasme is a perennial herb used in Mongolian medicine. Its extract has strong anticancer effect but has proven to be strongly toxic. To better understand how to reduce toxicity of its extract, we tested different extraction methods using a rapid and well validated laboratory fish model. In this experiment, alcohol, milk, yogurt, vinegar and myrobalan treatment - based extraction were used. Vinegar based extract was the most toxic. Even at 10,000 times and 100,000 times dilution zebrafish embryo showed 55% and 32.5% mortality respectively within 48h. The other extraction methods did not result in significant mortality. Exposure to all the extracts resulted in alterations of caudal fins particularly along the ventral margin where rays were missing. Cells in affected regions showed positive staining with TUNEL. The number of apoptotic cells of tail fin of zebrafish embryos increased from 3.3 in the control group to 9.6 -18.3 in the exposure groups. In the future we will place more emphasis on proliferation and apoptosis related genes and relevant protein expression.

1484 Supercritical Fluid Extraction and Characterization of Essential Oil from *Moringa oleifera* Seeds and Leaves Using GC/MS/MS

E. U. Obi. *Environmental and Interdisciplinary Sciences, Texas Southern University, Houston, TX.*

There has been a significant role played by plants in maintaining human health, and improving the overall quality of human lives for decades. Humans have used plants as traditional remedies to alleviate illnesses and promote the overall quality of lives due to the belief that plants have natural substances in them capable of promoting human health. *Moringa oleifera* has been shown through prior research to be a medicinal plant with a difference. It is a multipurpose tree used as spice, for cooking, as cosmetics oil and more importantly as medicinal plant. *Moringa oleifera* seeds and leaves cultivated in Nigeria was characterized for the presence of biologically active phytochemicals and antioxidant using supercritical CO2 extraction technique to extract the plant parts (seeds and leaves) and GC/MS/MS for structural elucidation of the compounds found. The supercritical CO2 extracts of *Moringa oleifera* seeds and leaves were screened for the presence of steroids, alkaloids, tannins, flavonoids, polyphenols, fatty acids and hydrocarbons. These compounds were found to be present in the extracts in varying proportions. The presence of these bioactive and therapeutically potent compounds make *Moringa oleifera* a therapeutically potent plant in which further study of the plant may lead to development of novel agents used to treat various disorders. Keywords: *Moringa oleifera*, phytochemicals, antioxidants, Supercritical CO2, GC/MS/MS, medicinal plant

1485 Antioxidant Potential of *Momordica charantia*

G. S. Iscan, S. Yilmaz, G. Coksari, O. B. Acikara, N. Artik and T. Coban. *Ankara University, Ankara, Turkey.* Sponsor: M. Iscan.

Momordica charantia plant has been reported to have possible antioxidant properties due to the rich content of phenolic compounds. In this study we determine the antioxidant potential of the seed and fruit of *Momordica charantia* different extracts by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and 2,2′-azino-bis(3-ethyl benzthiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assays. Regarding the scavenging capacity of the ABTS radical, the strongest activity was observed in the methanolic aqueous extract of fruit (IC50=15 µg/ml) followed by seed extract (IC50=39 µg/ml) among the tested extracts. IC50 values were calculated for all the extracts as well as trolox which was the reference standard for ABTS (IC50=1,5 µg/ml). However, lyophilized aqueous seed extract had (IC50=117 µg/ml) stronger antioxidant potential than that of fruit extract (IC50=213 µg/ml). According to DPPH scavenging assay, the strongest antioxidant activity of fruit was found in methanolic aqueous extract (IC50=260 µg/ml) followed by lyophilized aqueous extract (IC50=416 µg/ml). The highest antioxidant activity of seed was observed in lyophilized aqueous extract (IC50=342 µg/ml) followed by methanolic aqueous extract (IC50=465 µg/ml). IC50 values were calculated for all the extracts as well as BHT which is the reference standard for DPPH (IC50=11 µg/ml). It was generally observed that hexane extracts of seed and fruit had the weakest DPPH scavenging and ABTS radical cation decolorization capacity. These results show that the methanolic aqueous extracts of the seeds and fruits had more effective antioxidant capacities than the other extracts which could be due to their higher contents of gallic acid, protocatechuic acid, gentisic acid, catechin, chlorogenic acid and epicatechin than those of others.

1486 Effect of Impressic Acid on 6-Hydroxydopamine-Induced Neuronal Cell Death and Oxidative Stress

C. f. Zheng, S. W. Jin, Y. H. Kim and H. G. Jeong. *College of Pharmacy, Chungnam National University, Daejeon, Korea, Republic of.*

Impressic acid (IPA), 3α, 11α-dihydroxylup-20(29)-en-28-oic acid, is isolated from *Acanthopanax koreanum*, which has been used for many kinds of diseases, such as rheumatism, hepatitis, diabetes, and inflammatory disorders. However, little is known about the impact of IPA on the development of Parkinson's disease. This study is aimed to investigate the neuronal protective effects of IPA against parkinsonian mimetic 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in SH-SY5Y cells. Pretreatment with IPA significantly reduced 6-OHDA-mediated cell death and ROS generation. IPA increased expression of anti-oxidative enzymes, such as heme oxygenase-1 and NADPH quinineoxidoreductase-1. Furthermore, IPA increased the phosphorylation of Akt, ERK1/2, JNK1/2 and p38. These finding suggest that IPA has beneficial effects on the development of Parkinson's disease by down-regulating oxidative stress.

1487 Cardiotoxic Potential of 4-O-Methylhonokiol on Japanese Medaka (*Oryzias latipes*) Embryo-Larval Development

S. K. Singha, M. A. Ibrahim, M. Ilias, I. A. Khan and A. K. Dasmahapatra. *Department of BioMolecular Sciences, University of Mississippi, University, MS; National Center for Natural Product Research, University of Mississippi, University, MS.*

The present study was aimed to evaluate the teratogenic potential of 4-O-Methylhonokiol (4-O-MH), a neolignan isolated from *Magnolia grandiflora*, using Japanese medaka embryo-larval development as the experimental models. Fertilized medaka eggs (Iwamatsu stage 10) were exposed to 10 µM 4-O-MH for 2-, 4-, and 6 day post fertilization (dpf) and evaluated the heart beats, vessel circulation, thrombus formation, hatching efficiency and mortality. Heartbeats were determined on 3dpf and 6dpf embryos. It was observed that heartbeats were decreased significantly in all treatment groups both in 3- and 6dpf in comparison to their corresponding control groups. Moreover, thrombi were seen in embryos exposed to 4-O-MH both in 4 and 6 day groups. It was further observed that the vessel circulation was delayed significantly in the 4 and 6 days groups compared to the control groups. Moreover, treatment with 10 µM 4-O-MH for 4 days and 6 days prevent hatching; neither of the embryos from 4- and 6 days groups were able to hatch successfully. Exposure of this compound for 4- and 6 days also enhances mortality compared to the control and 2 day treatment groups. These preliminary data indicate that 4-O-MH might be a teratogen that has potential effect on the cardiovasculature and hatching and ultimately reduce the survivability of the embryos.

1488 Effect of Platycodin D on Heme Oxygenase-1 through Akt/Nrf2 Pathway in Endothelial Cells

G. H. Lee[1], Y. C. Chung[2] and H. G. Jeong[1]. [1]*College of Pharmacy, Chungnam National University, Daejeon, Korea, Republic of and* [2]*College of Public Health and Natural Science, International University of Korea, Jinju, Korea, Republic of.*

Platycodin D (PD) is a major triterpene saponin from the root of *Platycodon grandiflorum* A.DC, was reported to show pharmacological activities, such as ant-inflammatory and hepatoprotective properties. However, the anti-oxidative effect of PD and its underlying pathway remain unclear. This study investigated the protective effects of PD on Heme Oxygenase-1 in endothelial cells, EA.hy926. PD up-regulated HO-1 expression and Nrf2 nuclear-translocation in a dose- and time-

dependent manner. Also, PD increased the phosphorylation of Akt and MAPKs but pretreatment with Akt inhibitor (LY294002) only significantly inhibited PD induced-HO-1 expression and Nrf2 nuclear-translocation. These result suggest that PD could have vasoprotective effect by up-regulating the phase II enzymes via Akt/Nrf2 signaling pathways.

1489 Effect of Impressic Acid on Endothelial Nitric Oxide Synthase Activation in Endothelial Cells

S. J. Kim, S. W. Jin, Y. H. Kim and H. G. Jeong. *College of Pharmacy, Chungnam National University, Daejeon, Korea, Republic of.*

Nitric oxide produced by endothelial nitric oxide synthase (eNOS), key regulator of vascular functions, has anti-thrombotic and anti-atherosclerotic activities in the vasculature. This study investigated the intracellular pathways underlying eNOS activation by impressic acid (IPA). IPA, 3α,11α-dihydroxylup-20(29)-en-28-oic acid, is a lupane-type triterpenoid isolated from Acanthopanax koreanum, which has been used as a Korean folk medicine for rheumatism, hepatitis, diabetes, and inflammatory disorders. However, the anti-thrombotic and anti-atherosclerotic activities of IPA and its pathways remain unclear. IPA increased the phosphorylation of eNOS and the production of nitric oxide in a concentration- and time-dependent manner in human endothelial cells. IPA increased the phosphorylation of Akt, ERK1/2, JNK1/2, and p38. Furthermore, IPA increased the phosphorylation of AMP-activated protein kinase (AMPK), and calmodulin-dependent protein kinase II (CaMK II). Also, IPA-induced eNOS phosphorylation was blocked by respective inhibitors of Akt, MAPKs, AMPK, and CaMK II. These results suggest that IPA may have a possible potential of vasoprotective effect, stimulating eNOS phosphorylation and NO production via the activation of Akt, MAPKs, AMPK, and CaMK II.

1490 Myelostimulation by *Echinacea* Herbal Supplement and *E. purpurea* Independent of Bacterial LPS, Other Components That Activate Monocytes and Lipophilic Phytoconstituents

S. K. Nagumalli[3], H. F. Hussin[3], D. L. Barney[2], N. D. Pugh[1], D. S. Pasco[1] and S. A. Meyer[3]. *[1]National Ctr. for Natural Products Res., Univ. Mississippi, University, MS; [2]USDA-N. Central Regional Plant Intro. Station (NCRPIS), Ames, IA and [3]Toxicology, University of Louisiana-Monroe, Monroe, LA.*

Stimulation of myelopoiesis by *Echinacea* herbal supplement (Spring Valley Natural Whole Herb, Idea Sphere Inc., American Fork, UT) has been previously described by us (Ramasahayam et al. 2011. Planta Med. 77:1883-9). Ethanol extract (75%, v/v) was administered orally to Sprague-Dawley rats for 7 d at 50-100mg dry weight/kg/d, an amount comparable to label recommendation for human intake. This treatment yields a 70% increase in femur myeloid progenitors, i.e., CFU-GMs, from nucleated bone marrow cells upon culture in methylcellulose with csf2, IL-3 and SCF (Cameo assay, Hemogenix, Colorado Springs, CO). To determine that myelostimulatory activity was not due to supplement additives, dried plant source material (*E. purpurea*, aerial parts) was obtained (Ray Jaglowski, Twinlab Corp., Grand Rapids, MI), ethanol extracted (EtCP) and assayed for CFU-GMs. Further, since lipophilic N-alkylamides are major constituents of *Echinacea* spp., we also determined the bioactivity of n-hexane-washed EtCP (HxEtCP). Results showed that increase in CFU-GMs of EtCP was comparable to that of herbal supplement. Efficacy of myelostimulation by HxEtCP was equivalent to EtCP, but HxEtCP was 2.5X more potent than EtCP. Also, we tested two accessions of *E. purpurea* from the USDA N. Central Plant Introduction Station (Ames, IA) for myelopoeitic activity; PI-631307 and PI-633668 were found to increase CFU-GMs by 66% and 56%, respectively. Since *in vitro* activation of innate immune cells by *Echinacea* is known to be due to components of endophytic bacteria (Pugh ND et al. 2008. Int. Immunopharmacol. 8:1023-32), we determined LPS levels and monocyte stimulatory potential of the *Echinacea* extracts. LPS levels by the Limulus Amebocyte Lysate assay (Assoc. of Cape Cod) were 117 and 16.8 EU/g for EtCP and HxEtCP, respectively. EtCP and HxEtCP enhanced NFkB directed luciferase expression in THP-1 monocytes to levels 4.2% and 6.3% of LPS standard, respectively. Neither LPS nor components that activate monocytes correlated with myelostimulatory activity. In conclusion, *Echinacea* myelostimulatory activity is not due to LPS, other components that activate monocytes or plant lipophilics.

1491 Comparison of 1H-NMR Fingerprints of *Echinacea purpurea* Extracts with Stimulation of Myelopoiesis in Rat to Identify Active Constituents

H. F. Hussin[3], S. K. Nagumalli[3], T. W. Sasek[3], K. A. El Sayed[3] and S. A. Meyer[3]. *[1]Basic Pharmaceutical Sci., University of Louisiana-Monroe, Monroe, LA; [2]Sch. Sciences, University of Louisiana-Monroe, Monroe, LA and [3]Toxicology, University of Louisiana-Monroe, Monroe, LA.*

We have previously described stimulation of myelopoiesis by *Echinacea* herbal supplement (Spring Valley Natural Whole Herb, Idea Sphere Inc., American Fork, UT; E. purpurea aerial part) (Ramasahayam et al. 2011. Planta Med. 77:1883-9). A 70% increase in femur myeloid progenitors, i.e., CFU-GMs, upon culture in methylcellulose with csf2, IL-3 and SCF (Cameo assay, Hemogenix, Colorado Springs, CO) occurs with oral administration of a 75% ethanol extract for 7d at 50-100 mg/kg/d to female Sprague-Dawley rats. Comparable CFU-GM activity was obtained with ethanol extract of plant source material (EtCP) of the supplement (Ray Jaglowski, Twinlab Corp., Grand Rapids, MI). To assess whether activity was a general property of this species, we tested aerial parts of another *E. purpurea* sample (ULM) harvested from the campus of the University of Louisiana-Monroe (Long./Lat., 32.536728°,-92.068448°) for myelostimulatory activity. Treatment with ULM ethanolic extract (EtULM; 25-200 mg/kg/d) did not show a significant increase in CFU-GMs. Since Echinacea phytoconstituent class, N-alkylamides, and endophyte lipopolysaccharide (LPS) have been implicated as mediators of its immunological effects, we analyzed EtCP and EtULM for these constituents. LPS assayed with the Limulus Amebocyte Lysate assay (Assoc. of Cape Cod) was 117 and 83 EU/g for EtCP and EtULM, respectively. N-Alkylamides were assessed upon concentration in n-hexane washes (HxW) of EtCP and EtULM by 1HNMR (400 MHz, 20 mg/700 µl MeOH-d4) and HPTLC. 1HNMR spectral peaks with shifts equivalent to those of dodeca-2,4,8,10-tetraenoic acid isobutylamide (PhytoLab, Vestenbergsgreuth, Germany) were evident in HxW of EtULM, but absent from HxW of EtCP. HPTLC with apolar development confirmed that this constituent, as well as β-sitosterol and other lipophilic constituents were stripped from ethanolic extracts by n-hexane. Removal of n-hexane-soluble constituents did not unmasked a latent myelostimulatory activity of EtULM. Treatment with HxW of EtCP and of EtULM were without effect on CFU-GMs. In conclusion, these studies demonstrated that LPS or lipophilic constituents including N-alkyamides did not mediate or inhibit myelostimulatory activity of E. purpurea.

1492 Potential of Phytochemicals in Modulation of Cardiovascular Diseases and Breast Cancer Induced by Polycyclic Aromatic Hydrocarbons

S. Kondraganti and B. Moorthy. *Baylor College of Medicine, Houston, TX.*

Background: Heart disease and cancer are the leading cause of deaths in the United States. Environmental factors play a major role in the etiology of more than 80% of human malignancies. The environmental polycyclic aromatic hydrocarbons (PAHs) and the cytochrome P450s (CYPs) play an important role in the modulation of cardiovascular diseases (CVDs) and breast cancer. Dietary phytochemicals, curcumin and sulforaphane, possess potent antioxidant, anti-inflammatory and anti-tumor properties. Objective: 1) To study the cardio-protective and chemo-preventive abilities of the dietary phytochemicals (curcumin and sulforaphane). 2) To examine the potential of these phytochemicals to modulate the Phase I/Phase II enzyme systems and the pathways involved, thus inhibiting atherosclerotic lesion formation and cancer growth. Design/Methods: 21-day-old C57BL/6J mice were treated with the phytochemicals and then exposed to PAH, 7,12- dimethylbenz(a)anthracene (DMBA). Studies were also conducted by exposing C57BL/6J mice to DMBA and dietary phytochemicals, alone and in combination. The enzyme activities, protein, and mRNA expression of different genes isolated from hepatic, mammary, aorta and heart tissues of experimental mice were analyzed. Results: In hepatic, mammary, aorta, and heart tissues of mice exposed to DMBA, CYP1A1/1B1 (phase I enzymes) gene expression was increased significantly when compared to controls. Significant reduction in phase I gene expression was observed in mice treated with phytochemicals and DMBA. Increase in NQO1 (phase II enzyme) gene expression was observed in mice treated with DMBA. However, when the mice were treated with phytochemicals and DMBA, further increase in induction of phase II enzymes was observed. Highest levels of induction was observed when mice were treated only with phytochemicals and in combination. Curcumin and sulforaphane inhibited the PAH induced AHR gene expression, thus maintaining the cholesterol homeostasis. The phytochemicals were more effective when fed to mice before exposure to DMBA. Conclusions: The information gained from these studies will

help to determine whether the dietary phytochemicals can be given to humans daily as broad spectrum nutrient supplements to prevent different diseases. These studies will also aid in the design of more highly developed and effective prevention strategies, and treatment of CVDs and breast cancer involving dietary constituents.

1493 Amoebicidal Activity of Extracts of *Rhus trilobata* on *Entamoeba histolytica* Trophozoites

H. A. García-Ramírez[1], M. d. C. González-Horta[1], R. Infante-Ramírez[1], M. R. Hernández-Castaños[1], P. Talamás-Rohana[2] and B. E. Sánchez-Ramírez[1]. [1]*Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua, Chihuahua, Chih., Mexico and* [2]*Infectómica y Patogénesis molecular, Centro de Investigación y Estudios Avanzados, México, D.F., Mexico.* Sponsor: L. Mía. del Razo Jiménez.

In Mexico, intestinal amoebiasis morbidity is estimated in 5,000 cases per 100,000 people, being children the most affected. Metronidazole (MTZ) is considered the antiamoebic drug of election, being the most used for the treatment of luminal and extraluminal infection. Secondary adverse effects of long-time MTZ administration included cytotoxic and genotoxic effects as well as carcinogenic risk; in addition, amoebas might develop resistance to these drugs. The objective of this work was to evaluate the amoebicidal activity of the plant extract *Rhus trilobata* on *Entamoeba histolytica* trophozoites. With this purpose, steams and fruits extracts of *Rhus trilobata* were prepared by boiling in water (AE) or by maceration in 70% methanol. Solid phase separation using C18 columns and solvents of different polarity were used for separation of stems extracts. Viability tests of cells treated with the obtained fractions were performed using the WST-1 reagent; concentrations tested were 0, 2.5, 5 and 10 µg/mL of each extract. Results showed that crude AE, and both A2 and A4 fractions, had a significant decrease on trophozoites viability compared with control cells without treatment; the effect of A2 and A4 fractions at 10 µg/mL had no difference with MTZ IC50 (0.04 µg/mL). Methanol extract or their fractions had no significant effect on trophozoites viability at concentrations tested. Both extracts of fruits, aqueous and methanolic, decreased significantly the cell viability in a dose-response, by an IC50 of 17.13 y 17.17 µg/mL, respectively. These results demonstrated that *Rhus trilobata* extracts contain compounds that have an important amoebicidal effect. Additional studies are being conducted to elucidate the nature and mechanism of these active ingredients. (This work was partially supported by FOMIX CHIH-2010-C01-147532).

1494 General Composition and Antifungal Effect of Commercial Ethanolic Mexican Propolis on *Aspergillus flavus*

R.-U. Antonio[4], M.-A. GCG[1], M.-A. A[2], T.-E. RS[4] and M.-C. AG[4]. [1]*Faculty of Agronomy, UANL, Gral Escobedo, NL, Mexico;* [2]*Multidisciplinary Research Unit, Faculty of Superior Studies, Cuautitlan, UNAM, Edo México, Mexico;* [3]*Toxicology, Faculty of Veterinary Medicine, UANL, Gral Escobedo, NL, Mexico and* [4]*Veterinary Toxicology, Faculty of Veterinary Medicine, UANL, Gral Escobedo, NL, Mexico.*

Propolis is a natural resinous substance collected by bees and majorly used as beehive sealant. As the result of multi-chemical composition, propolis has many biological properties that can benefit human and animal health, such as its potential use to control fungal contamination in foods. In this research we determined the general composition and the *Aspergillus flavus* antifungal effects of 10 commercial ethanolic extracts collected in Mexico. Total resin, protein (Bradford assay) and polyphenol content (Folin-Ciocalteu reagent) were measured to evaluate propolis composition. *A. flavus* inhibition of radial growth assay was used to evaluate its antifungal effectiveness. Briefly, fungal buttons (0.5 mm diameter x 2 mm thick) of *A. flavus* culture were placed in the center of petri dishes prepared with propolis media (propolis 0, 0.5, 1, 2.5 and 5% in Sabouraud media) and placed on an incubator at 32 C for 5 days. Inhibition of growth was measured with a ruler and recorded in addition, spore suspensions at 5 day culture of all treatments was evaluated by using a Neubauer's chamber (haemocytometer) on tween media. Data was analyzed with parametric assays of Analysis of Variance (One-Way ANOVA), and the comparisons between the means were performed using the multiple comparison test of Tukey, with a significance level of 5% ($P \leq 0.05$). Results showed moderate variations of propolis composition. Ranges of resin content were 370-900 mg/mL, while protein and polyphenol ranges were 0-3.8 mg/mL and 90-2270µg/mL, respectively. Variations were also noted in antifungal effectiveness where inhibition of growth varied from 0.5, 1 % as the lowest effect and 2.5 %as the minimal effective concentration, in the spore count the 2.5 and 5% are the most effective concentration and 1% and lower concentration haven't differences with the 0% treatment. Clearly the effectiveness of propolis as antifungal can be influenced by its chemical composition. Funding: CONACYT-SNI 56078. Key words: propolis, *Aspergillus*, antimicotic.

1495 Compounds and Fractions from *Oxyanthus speciosus* Showed Good *In Vitro* Antimycobacterial Activity and Low Cytotoxicity Profile

A. O. Aro, M. D. Awoufack, J. N. Eloff and L. J. McGaw. *Paraclinical Sciences, University of Pretoria, Onderstepoort, South Africa.*

Tuberculosis, a disease caused by *Mycobacterium tuberculosis* has re-emerged at an alarming rate, becoming a global concern due to the upsurge of HIV/AIDS and resistant strains, remains a threat to the health of human. TB was reported to cause 1.4 million mortality of the world's population in 2012 and an estimated 8.7 million death. The incidence, prevalence and mortality rates of TB have reduced in the Western world but still remain alarming in Asia and Africa. The upsurge of resistance strains has rendered the available drugs ineffective. The use of medicinal plants is an alternative to the use of synthetic drugs as a curative agent for infectious disease, arousing the interest of researchers investigating substitutes to modern medicine. The acetone extract, fractions and 2 compounds from *Oxyanthus speciosus* DC. were screened for their antimycobacterial activity against 3 non-pathogenic mycobacteria namely: *Mycobacterium aurum*, *Mycobacterium fortuitum* and *Mycobacterium smegmatis* and a pathogenic mycobacteria: *Mycobacterium tuberculosis* (8104) using a microdilution assay. Bioautography was used to determine the presence of antimycobacterial compounds of the plant extracts and fractions. Cytotoxicity was determined using the tetrazolium-based colorimetric cellular assay (MTT) against C3A human liver cells and Vero kidney cell. The selectivity index (SI) values of the extracts were calculated. The extract had a significant activity against the 4 tested organisms with a minimum inhibitory concentration (MIC) values ranging from 60 - 170 µg/ml. Two fractions out of 11 had significant activity against *M. smegmatis* with MIC value of 39µg/ml. Compound 1 showed a moderate activity against all the tested mycobacteria strains with MIC values ranging from 12.5 - 50 µg/ml. The acetone leaf extracts of *O. speciosus*, and 4 fractions had relatively low cytotoxicity with LC50 value ranging from 0.160 - 0.383 mg/ml against C3A human liver cells. Compound 1 showed no cytotoxicity against Vero kidney cell even at the highest concentration (200 µg/ml) while compound 2 had an LC50 value of 33.77 µg/ml. The crude extract and all the fractions had selectivity index (SI) values (LC50/MIC) ranging from 0.03-3.27. Compound 1 had the best SI values ranging from 4-16. The promising activity of compound 1 *in vitro* suggests its potential as an anti-TB drug candidate.

1496 Nano-Scaled Cerium Oxide Induces Platelet Activation *In Vivo*

D. C. Davidson[2], M. Barger[2], R. Derk[2], T. A. Stueckle[2], J. Ma[2], J. Cohen[1], P. Demokritou[1] and L. Wang[2]. [1]*Department of Environmental Health, Harvard School of Public Health, Boston, MA and* [2]*Health Effects Lab Division, Allergy and Clinical Immunology Branch, CDC/NIOSH, Morgantown, WV;* [3]*Health Effects Lab Division, Pathology and Physiology Research Branch, CDC/NIOSH, Morgantown, WV.*

Nano-scaled cerium oxide (nCeO2) is used in a variety of applications, including use as a fuel additive, catalyst, and polishing agent, yet potential adverse health effects associated with nCeO2 exposure remain incompletely understood. *In vivo* studies using a rat model have shown that inhaled nCeO2 can deposit in deep lung tissues and induce fibrosis; however, little is known about other potential cardiovascular disorders (CVD) associated with nCeO2 exposure. Similarly, blood-based biomarkers to predict risk of nCeO2-induced disease remain limited. To address these knowledge gaps, rats were intratracheally instilled with 3.5 mg/kg nCeO2 and plasma samples were analyzed 28 days after treatment to identify potential biomarkers of such nCeO2 -induced disorders. Plasma samples revealed increased levels of pro-inflammatory mediators such as IL-1β and IL-6, as well as the pro-fibrogenic mediator TGFβ1 in response to nCeO2. Platelets are one of the main sources of circulating TGFβ1, and these small (3-5 µm), anucleate cells are known to contribute to numerous inflammatory disorders and CVD following activation and subsequent degranulation. Thus, we hypothesized that platelets are activated in response to nCeO2 treatment. Consistently, we found that platelets isolated from nCeO2-treated rats released significantly more TGFβ1 than those isolated from control animals, indicating degranulation of these cells and suggesting that platelets may be contributing to nCeO2-induced fibrosis and CVD. Interestingly, an amorphous silica-coated nCeO2 (amsCeO2) induced levels of TGFβ1 from platelets

consistent with those isolated from saline-treated animals, demonstrating that "safety-by-design" strategies for preparing engineered nanomaterials may prove useful in ameliorating some of the harmful effects of nCeO2. Collectively, these results shed light on possible biomarkers for nCeO2-incuded disease and highlight a potential role for platelets in such diseases. Disclaimer: The findings and conclusions in this abstract are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

1497 Animal Inhalation Exposure to Nanomaterials: Design, Conduct, and Data Interpretation

B. T. Chen. *NIOSH, Morgantown, WV.* Sponsor: P. Joseph.

With the dramatic growth of nanotechnology, there are concerns about potential health risks of these man-made nanoparticles in workplaces. These particles possess a large specific surface area which can enhance their reactivity on cellular surfaces as well as small size that allows translocation across cellular barriers once deposited in the lungs. Toxicological studies have been conducted by using laboratory animals exposed to aerosols containing nanoparticles via inhalation. The design of an adequate animal exposure is based on human equivalent dose in an occupational environment. However, physical properties of airborne nanoparticles in an exposure chamber are sometimes difficult to determine because their size and concentration may be different from those of particles in the bulk material due to aggregation. In addition, particle loss onto chamber surfaces due to diffusion and electrostatic charges affects the uniformity in the chamber. It is thus important to investigate the dispersion property and aerodynamic behavior of test nanomaterials prior to conducting the exposures. In this study, three nanomaterials, titanium dioxide (TiO2), carbon nanotubes (CNT), and cerium oxide, were respectively selected to mimic real-life aerosols with a diverse range of particle morphologies. They were used to demonstrate that different nanomaterials may require different exposure designs. Results indicated that, not only different generation (e.g., powder dispersion, liquid nebulization, *in situ* flame reaction) and characterization (e.g., microscopic counting, gravimetric analysis, real-time monitoring) methods should be selected to complement the diverse physical characteristics of the particles, but analysis in terms of exposure metric (count, surface area, or mass) and dose relevancy (e.g., metric, target, and response) should be carefully dealt with depending on the biological endpoints of the studies. For example, an acoustic vibration setup with a gravimetric tool was adequate for fibrous CNT exposure, while a magic-finger activation system with a number-based monitoring device was suitable for spherical TiO2 exposure. In summary, the information provided here will help researchers effectively design and conduct animal inhalation exposure studies that yield useful toxicological data of nanomaterials.

1498 A Framework for the Derivation of a Health-Based Occupational Exposure Limit for Silver Nanoparticles

B. A. Weldon[3], E. M. Faustman[3], G. Oberdörster[2], T. Workman[3], W. C. Griffith[3], C. Kneuer[1] and I. J. Yu[4]. [1]*Federal Institute for Risk Assessment, Berlin, Germany;* [2]*Environmental Medicine, University of Rochester, Rochester, NY;* [3]*Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA and* [4]*Institute of Nanoproduct Safety Research, Hoseo University, Asan, Korea, Republic of.*

With the increased production and widespread commercial use of silver nanoparticles (AgNPs), human and environmental exposures to AgNPs are inevitably increasing. In particular, persons manufacturing and handling AgNPs and AgNP containing products are at risk of exposure, potentially resulting in health hazards. While silver dusts, consisting of micro-sized particles and soluble compounds already have established occupational exposure limits (OELs), silver nanoparticles exhibit different physicochemical properties from bulk materials and an additional OEL may be needed. Our framework integrates toxicological and exposure data newly available for AgNPs to determine potential health risk to workers and to derive an OEL. Our approach considers toxicokinetic factors and is adjusted for silver dosimetry and clearance. Our proposed OEL for AgNPs is based on a dosimetrically adjusted benchmark concentration (BMC) calculated from subchronic rat inhalation toxicity assessments, and estimated human equivalent concentration (HEC). It is anticipated that our recommended level will protect workers from potential health hazards, including lung, liver, and skin damage. This work was done in collaboration with the NCNHIR Consortium and supported by NIH/NIEHS grants U19ES019545, P30ES07033 and RD835.

1499 Effects of CdSe/ZnS Quantum Dots on Reactive Oxygen Species and Respiratory Burst in Peripheral Blood Leukocytes Taken from Gclm Wild-type, Heterozygous and Null Mice

C. C. White[2], D. Botta[2], C. M. Schaupp[2], D. K. Scoville[2], X. Gao[2] and T. J. Kavanagh[2]. [1]*Bioengineering, University of Washington, Seattle, WA and* [2]*Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*

CdSe/ZnS quantum dot (QD) nanoparticle exposure is associated with the production of reactive oxygen species (ROS), possibly as a consequence of compromised mitochondrial respiration, or activation of NADPH oxidases (NOX), either of which can result in enhanced superoxide anion radical (O2-.) levels. Neutrophils and monocytes are known to produce O2-. after stimulation with bacterial and fungal components, or with phorbol ester (PMA), which involves activation of a NOX-dependent respiratory burst. It is unknown what effect QDs have on ROS levels and NOX activation in peripheral blood leukocytes, and whether this is influenced by glutathione (GSH), which is dependent on glutamate cysteine ligase modifier subunit (Gclm) expression. In this study we evaluated the effects of QDs on ROS levels under basal and PMA-stimulated conditions in peripheral blood leukocytes from Gclm wild-type (WT), heterozygous (Het) and null (KO) mice. Leukocytes were incubated with QDs for 3 hr at increasing concentrations (0.25 nM - 5 nM), and basal and PMA-stimulated ROS levels in leukocyte subsets were measured using DCFDA fluorescence and flow cytometry. At low level exposure, QDs caused an increase in baseline ROS levels in blood neutrophils (but not monocytes) from Gclm KO and WT mice, but at higher QD concentrations (above 1 nM) there was depression of this effect. QDs caused inhibition of PMA stimulated NOX activity in neutrophils taken from Gclm WT and KO mice (but not Het mice). There was no appreciable effect of QDs on NAD(P)H levels, as indicated by UV light-induced blue autofluorescence. These data suggest that QD-mediated increases in ROS levels, with simultaneous inhibition of NOX activity may result in compromised neutrophil function and innate immunity, especially in cases of GSH deficiency. Support: NIEHS grants U19ES019545, P30ES007033, T32ES015459, T32AG000057.

1500 Different Surface Coatings on IONP Project Differential Proteomic Patterns of Protein Corona

V. Escamilla-Rivera[2], A. Solorio-Rodriguez[2], M. Uribe-Ramirez[2], R. Winkler[1] and A. De Vizcaya-Ruiz[2]. [1]*Departamento de Biotecnologia y Bioquímica, Cinvestav-Irapuato, Irapuato, Mexico and* [2]*Departamento de Toxicología, Cinvestav, Distrito Federal, Mexico.*

Protein corona (PC) has been proposed as the main biological entity of cell interaction that could define the toxicological response to Fe3O4 nanoparticles (IONP). Coating of IONP with polyethilenglycol (PEG) and polyvinylpyrrolidone (PVP), is a widely accepted strategy to avoid excessive protein binding and undesirable uptake. Previously we demonstrated that IONP coated with PEG induced complement activation and proinflammatory response in a rodent model i.v. administrated. This study aimed to identify the protein profile of the PC on IONP with different surface coatings. Blood plasma was employed as a source of proteins in order to form a hard PC on IONP bare (IONP-b), coated with PVP (IONP-PVP) and PEG (IONP-PEG). After PC formation, proteins were desorbed from IONP, resolved in a SDS-PAGE and identified by LC-MS-MS. Protein analysis was performed using Gene Ontology tools. In total, 409 proteins were identified adsorbed to IONP-b, 497 on IONP-PVP and 434 on IONP-PEG. A set of 263 proteins was found to be shared by all three IONP and account for 64%, 52% and 60% of IONP-b, PVP and PEG of total coronas, respectively. Proteins involved in response to an external stimulus, lipid transport, coagulation and complement system were mainly adsorbed to IONP-b. IONP-PVP showed less number of proteins involved in lipid transport but more proteins related to coagulation compared to IONP-b. Meanwhile PEG coating adsorbed less proteins associated to lipid transport and coagulation, and more related to the immune response compared to IONP-b. These results suggest that only a small particular set of proteins surrounding the IONP-PEG could impair homeostasis and be involved in inducing a pro-inflammatory response and complement activation *in vivo*. These findings could be used to perform selective protein targeting coatings to be used to avoid triggering of adverse effects to IONP.

1501 Integrated 'Omics' of Coelomic Fluid: An Attempt to Understand Phenomics of TiO2 Nanoparticle Toxicity

S. P. Bitragunta and S. G. Palani. *Department of Biological Sciences, Birla Institute of Technology and Science Pilani, Hyderabad Campus, Hyderabad, India.*

Background: Toxicological evaluation of TiO2 nanoparticles (TNP) has gained prominence across the globe to promote their environmental and health safety. Despite progress in assessing ecotoxicity of (TNP), mechanistic aspects of the same are unclear. Moreover, standard methods of toxicity testing in sentinel organisms, dose-response metrics in ecotoxicology studies are inadequate to substantiate TNP exposure in the environment. In this context, high-throughput methods which can facilitate ecotoxicological evaluation of nanoparticle are inevitable in environmental monitoring studies. Methods: Physicochemical characterization of TNP and integrated 'omics' approach that incorporate proteomics and metabolomics were used to divulge toxic effects of TNP on earthworm, *Eisenia foetida*. Earthworms were exposed to TNP of concentration from 0.05 to 0.25 mg/ml as per OECD-207 guidelines. Characterization and quantification of TNP were carried out by dynamic light scattering and ICPMS respectively. LCMS-based proteomics and metabolomics approach was used to screen biomarkers related to amino acid metabolism and oxidative stress in coelomic fluid of earthworms exposed to TNP. Results: It was found that pH of the dispersion medium influenced the agglomeration behavior of TNP. Multivariate statistical analysis of 'omics' data revealed that TNP could alter the levels of amino acids including L-alanine, L-aspartate, L-methionine, S-adenosylmethionine and L-lysine thereby affecting oxidative stress in the earthworm. TNP exposure led to the differential expression of heat shock proteins and metallothioneins in coelomic fluid. Conclusions: Results substantiated the importance of coelomic fluid as a complimentary biological medium that can help in delineating TNP toxicity. TNP could trigger sub-lethal effects in earthworm by altering the levels of amino acids and proteins thereby metabolic pathways. Integrating 'omics' assisted in identifying the plethora of metabolites that can significantly fluctuate during earthworm exposure to TNP. Outcomes of the study will definitely assist in paraphrasing subtle responses that are vital in understanding the phenomics of earthworm exposed to nanoparticles.

1502 Cholesterol-Induced Phenotypic Alterations in Cellular Response to Nanoparticles

J. H. Shannahan, V. C. Minarchick and J. M. Brown. *Department of Pharmaceutical Sciences, University of Colorado, Aurora, CO.*

Nanoparticles (NPs) are being synthesized for biomedical applications, which require their direct introduction into the circulatory system. A significant and growing portion of the population exists with high circulating levels of low-density lipoprotein (LDL). This physiological environment high in LDL is known to induce phenotypic changes in macrophages and endothelial cells allowing for disease progression. These alterations include increased surface expression of scavenger receptors (SRs) that facilitate both the uptake of LDL and NPs. Therefore we hypothesize that the presence of LDL will result in macrophage and endothelial cell phenotypic alterations resulting in altered cellular-NP interactions and toxicity. Macrophages (RAW264.7) and rat aortic endothelial cells (RAEC) were grown in either media without LDL present or with LDL at 25mg/dl. Alterations in cell surface expression of SR-BI, SR-AI, and MARCO were assessed by flow cytometry. The presence of LDL resulted in increased expression of all SRs evaluated, of which SR-BI was the most induced. Cells grown in these conditions were exposed to increasing concentrations (0, 6.25, 12.5, 25, or 50µg/ml) of 20nm silver NPs (AgNPs), 100nm AgNPs, or 20nm Fe3O4 NPs and no differences in cytotoxicity were determined through 3h. Following a 2h exposure to NPs at 50µg/ml, cells grown in LDL-rich media were found to internalize fewer NPs compared to cells grown in the absence of LDL. Lastly, cell activation was analyzed following a 1h exposure to NPs at 50µg/ml. Cells grown in LDL-rich media, that overexpressed SRs, induced a greater inflammatory response as measured by IL-6 mRNA expression following NP exposure. General inhibition of SRs by dextran sulfate resulted in decreased NP uptake in both environments as well as a reduction in the inflammatory response of cells in the LDL-rich environment. These findings demonstrate the possible impact of disease-related cellular phenotypic alterations on NP toxicity thus influencing their utility for biomedical applications. This work was supported by NIEHS K99/R00 ES024392.

1503 Topical Skin Treatment with Nanoparticles Modulates the Contact Hypersensitivity Response in a Murine Model

B. C. Palmer[2], S. Jatana[2] and L. DeLouise[2]. [1]*Biomedical Engineering, University of Rochester, Rochester, NY and* [2]*Toxicology, University of Rochester, Rochester, NY.*

Nanoparticle engineering is a growing field due to the vast array of applications. For example, titanium dioxide and zinc oxide nanoparticles are included in a number of sunscreens for their ultraviolet light filtering properties, and silica based nanoparticles and carbon nanotubes are being researched as potential drug delivery vehicles through skin. There is a need to test the dermal toxicity of these nanoparticles, and since skin is exposed to a number of potential allergens we find it equally important to examine the effect of nanoparticle application on immune system activation after allergen exposure. Here we examine the Type IV immune response in the contact hypersensitivity (CHS) model after sensitization and challenge with dinitrofluorobenzene (DNFB). We have found that unmodified 20 nm silica nanospheres reduce the swelling response in the contact hypersensitivity model. The swelling is only reduced when the silica nanospheres applied up to one hour before or one hour after the chemical hapten challenge, and the response appears to be related to the size and surface charge of the nanoparticles. Importantly, we have also identified a reduced swelling response when silica nanoparticles are co-administered along with 2-deoxyurushiol, another skin irritant that resembles the chemical structure of the allergens in poison-ivy. In search of a potential mechanism, we have found both reduced inflammatory cytokine (IL-6, IL-1β, KC, MIP-2) levels and reduced mast cell degranulation when 20 nm silica nanospheres are administered along with DNFB in the challenge phase. Interestingly, we have found opposite trends in skin swelling and inflammation when carboxylated multi-walled carbon nanotubes (30 nm diameter, 5-20 µm length) are administered along with DNFB in the challenge phase. Future work will include continued research into mechanisms of action and exploration of nanoparticles as a potential preventative treatment for contact dermatitis, but will also focus on carbon nanotubes since they exacerbate CHS responses, and represent an interesting toxicological concern due to their increased use in biomedical science.

1504 A Computational Framework for Interspecies Pharmacokinetics, Exposure and Toxicity Assessment of Gold Nanoparticles

Z. Lin[3], N. A. Monteiro-Riviere[3], R. Kannan[1] and J. E. Riviere[3]. [1]*Department of Radiology, University of Missouri, Columbia, MO;* [2]*Institute of Computational Comparative Medicine, Kansas State University, Manhattan, KS and* [3]*Nanotechnology Innovation Center of Kansas State, Kansas State University, Manhattan, KS.*

Interspecies and *in vitro* to *in vivo* extrapolation of pharmacokinetic and toxicology data is crucial for successful translation from laboratory studies to humans and for proper risk assessment of nanomaterials. This manuscript reports a comprehensive computational framework built on physiologically based pharmacokinetic (PBPK) modeling that successfully simulated tissue distribution of gold nanoparticles (AuNP) across a wide dose range and several species, including mice, rats, pigs, and humans. This framework was evaluated with multiple independent datasets, demonstrating excellent predictive capability. Cross-species extrapolation evaluation was done from mice, rats, and pigs, respectively, to humans. The simulation results suggest that rats and pigs seem to be more appropriate models than mice in species extrapolation of AuNP pharmacokinetics and that the dose and age should be considered in this extrapolation. These results partially explain the current low translation rate of nanotechnology-based drug delivery systems from mice to humans. Also, it provides a scientific basis for researchers to select the most appropriate animal model and dosing paradigm for conducting future nanomaterial studies to increase the research relevance to humans. This framework showed that by incorporating *in vitro* and/or *in vivo* cellular uptake and toxicity data into PBPK models, risk assessment and interspecies extrapolations can be improved. This simulation approach may be applied to other types of nanomaterials and provide guidance to the design of future translational, pharmacokinetic, and toxicological studies. (Supported by the Kansas Bioscience Authority)

1505 Identifying the Effects of Size and Shape on the Physicochemical Properties of Cerium Oxide Nanoparticles

C. M. Sims, J. M. Gorham, T. J. Cho, I. Levin, V. A. Hackley and B. C. Nelson. *Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD.*

Recently, the use of cerium oxide (CeO2, ceria) nanoparticles (NPs) has grown extensively due to their current and potential applications, such as industrial catalysts and biomedical agents. While the physicochemical characteristics (e.g. size, shape, surface chemistry) of ceria NP are known to highly influence their performance for a given application, the impact of these characteristics on their environmental and toxicological fates is not yet fully understood. Previous research designed to understand the potential environmental and toxicological effects of ceria NPs have yielded conflicting results, with ceria NPs found to be both toxic and non-toxic to cells and organisms. The mixed results of these studies reinforce the need to better understand the physicochemical characteristics of ceria NPs and how these characteristics affect their potential mechanisms of toxicity. Here, we utilize multiple analytical techniques, including powder X-ray diffraction (XRD), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), electron-energy loss spectroscopy (EELS), dynamic light scattering (DLS), and ultraviolet-visible spectroscopy (UV-Vis) to thoroughly characterize a suite of ceria NPs, both as dry powders and in environmentally relevant media. Preliminary data suggest the primary particle size has a large effect on the oxidation state of the ceria NPs, with smaller particles appearing to have increased Ce3+/Ce4+ ratios compared to their larger counterparts. Attempts to create stable aqueous suspensions of the studied ceria NPs were unsuccessful unless their surfaces were modified by various ligands (e.g. citrate, PVP). Additional experiments suggest the identity and amount of surface coating on the ceria NPs highly impacted their resultant dynamics in solution. Imminent work will investigate the potential toxicity of ceria NPs as a function of their physicochemical characteristics and the resultant effects on uptake, distribution, and potential mechanisms of toxicity in cells and organisms.

1506 *In Vitro* Aerosol Exposure System for Nanotoxicity: Dosimetric Analysis Using Nanometals and Nanometal Oxides

T. B. Tilly, J. J. Schlager and S. M. Hussain. *Molecular Bioeffects Branch, Bioeffects Division, Human Effectiveness Directorate, 711th Human Performance Wing/RHDJ, Air Force Research Laboratory, Wright-Patterson Air Force Base, OH.*

"The dose makes the poison" has been the fundamental principle of toxicology since its scientific inception, but despite this principle, there are many *in vitro* nanotoxicology studies to date that have failed to consider the delivered cellular dose of nanomaterials (NMs). For experimental execution, the multitudes of natural and man-made NMs frequently prove to possess solubility issues combined with the difficulties in creating a liquid dispersion make it problematic to draw comparisons between current published toxicity studies. Additionally, there is a need for more enhanced *in vitro* methods that evaluate the toxicity of NM deposited at the air-liquid interface of the respiratory tract. Aerosol exposure chambers (AECs) have been developed to expose NMs to cell cultures at the air-liquid interface; however, dosimetry of NMs using these chambers requires improvement. Currently, most studies published provide a determination of dose estimated prior to the exposure using electron microscopy and gravimetric analysis techniques; however, these approaches do not take in account the complex nature of aerosols and the inconsistency of deposition makes determining the actual respiratory system regional delivered dose quite difficult. In this study, we dispersed nanopowders of ZnO, CeO2, NiO, and Ag in air and exposed these individually to nasal and lung cells using our AEC design. Cellular viability and inflammation (IL-8) were determined at 1, 4, and 24 hrs after exposures. Dosimetry was predicted using the Fusch and Gunn theory of aerosol data collected from the optical and scanning mobility particle sizers, and the delivered dose was determined using inductively coupled mass spectrometry. All cell cultures remained viable for the entire study; however, significant induction of the inflammatory marker IL-8 was observed 24 hrs after exposure in the lung and nose epithelial cells exposed to Ag, ZnO, and NiO.

1507 Iron-Doped Apatite Nanoparticles Increase Bacteriophage Infectivity and Macrophage Bioactivity

K. L. Trout[3], J. M. Andriolo[3], A. Holian[3], M. L. Pedulla[4], M. K. Hailer[4] and J. L. Skinner[4]. [1]*Bioengineering, Individualized Interdisciplinary Doctoral Program, University of Montana, Missoula, MT;* [2]*Biological Sciences, Montana Tech, Butte, MT;* [3]*Center for Environmental Health Sciences, University of Montana, Missoula, MT and* [4]*Chemistry and Geochemistry, Montana Tech, Butte, MT;* [5]*General Engineering, Montana Tech, Butte, MT.*

Bacteriophages (phages) are viruses that infect and eliminate bacteria as a means of replication. Although research in the early 1900s focused on phages for antibacterial therapy, interest dwindled in the western world during the advent of antibiotic drugs. However, there has been a recent resurgent interest in phage therapy due to increasing antibiotic resistance issues in the medical, veterinary, and food industries. This study presents a unique observation that specifically synthesized iron-doped apatite nanoparticles (IDANPs) enhance phage bactericidal activity. IDANPs were synthesized, purified, and characterized. Staphylococcus aureus were exposed to IDANPs prior to phage infection and antibiotic activity was evaluated by quantification of death zones (plaques). Increased plaque formation was dependent on the iron concentration of the nanoparticles. No change was observed after exposure to control particles of various compositions. These results suggested that IDANPs may be useful as a phage therapy adjuvant. To further evaluate IDANP therapeutic potential, macrophage bioactivity in response to IDANP exposure was measured. Macrophages were selected because they are involved in particle phagocytosis and mediation of inflammation. Primary C57Bl/6 mouse alveolar macrophages were exposed to IDANPs *in vitro* and were assessed by MTS assay, LDH assay, and ELISA for production of IL-1β, IL-6, and TNF-α. IDANPs were cytotoxic, but did not induce release of these pro-inflammatory cytokines. While these toxicity results suggest IDANPs in their present form may not be a safe therapeutic, ongoing studies aim to understand the mechanism of IDANP-mediated increase in phage infectivity, which may lead to development of a more biocompatible therapy. In addition, the potential of foreign material exposure to increase infections of other viruses is a relatively unexplored area of research that is worth considering. This work is supported by P30GM103338, R01ES023209, and CBET-1338478. Content is solely the responsibility of the authors and does not necessarily represent official views of NIH or NSF.

1508 Particulate Matter Releases From Nano-enabled Products Across Their Life Cycle (LCPM): an Integrated Methodology Linking LCPM Exposures to Hazard

J. M. Cohen, A. K. Pal, C. Y. Watson, G. M. DeLoid and P. Demokritou. *Harvard School of Public Health, Boston, MA.*

Increasing consumer exposures to products and materials with nanoscale components has raised concerns over potential hazards to human health and the environment. Complex particle mixtures may be released from nano-enabled products (NEPs) across various stages of their product life cycle (LCPM). Characterization of these releases during typical end of life scenarios (wear and tear, weathering, incineration, etc) is necessary to accurately characterize the risk implications for human health. Here we present an integrated methodology for sample collection, extraction, dispersion and characterization of relevant consumer-level exposures to real world LCPM. In addition, recently developed integrated dosimetric tools are also applied in order to bring *in vitro* and *in vivo* toxicological doses onto the same scale. This multistep methodology consists of: (1) real-time monitoring and sampling of size fractionated LCPM; (2) extraction of collected LCPM using aqueous or ethanol extraction protocols to ensure minimal physicochemical alterations; (3) optimized LCPM dispersion preparation and characterization; (4) use of dosimetric techniques for *in vitro* and *in vivo* toxicological studies. The proposed methodology was then used to evaluate two "real world" LCPM systems simulating consumer use and disposal of NEPs: 1) LCPM generated from consumer use of printer toners embedded with nanoscale particles, and 2) LCPM generated via thermodecomposition of composite materials consisting of polyurethane embedded with carbon nanotubes. The LCPM for each scenario were collected and characterized for physical-chemical properties, and applied to small airway epithelial cells for *in vitro* toxicological evaluation at doses relevant to expected consumer level exposures. Cellular toxicity was observed only at doses that are several fold higher than consumer level concentrations characterized in step 1. This comprehensive framework provides a standardized methodology to link relevant consumer level exposure concentrations of LCPM to toxicity measured *in vivo* and *in vitro*, thereby addressing important knowledge gaps in the field of nanotoxicology.

1509 Proteomic Profiling of Protein S-Glutathionylation Reveals Selective Redox Regulation of Macrophage Pathways During Nanoparticle-Induced Oxidative Stress

B. D. Thrall, J. Duan, V. Kodali, M. Gaffrey and W.-J. Qian. *Pacific Northwest National Laboratory, Richland, WA.*

Oxidative stress is thought to be a major mechanism of toxicity for engineered nanoparticles (ENPs), yet the precise intracellular targets involved remain poorly understood. We investigated protein S-glutathionylation (SSG) as a redox-dependent mechanism by which ENPs may alter macrophage innate immune functions. A novel quantitative redox proteomics approach for site-specific identification of SSG permitted us to quantify, for the first time, the specific intracellular protein targets and pathways susceptible to redox modifications following ENP exposure in a model macrophage cell (RAW 264.7). Three high-volume materials (SiO2, Fe3O4 and CoO) were used as representative ENPs with low, moderate, and high propensity to stimulate cell reactive oxygen species (ROS) and disrupt macrophage function. The total number of SSG modifications identified correlated well with the overall level of ENP-induced cellular redox stress and impairment of macrophage phagocytic function against bacteria (CoO > Fe3O4 > SiO2). Moreover, pathway-specific differences in SSG modifications were observed between ENPs which induce low versus high levels of redox stress. Pathways regulating phagocytosis, protein translation, and protein stability were significantly enriched with SSG-modified proteins in response to ENPs that induce sub-cytotoxic levels of redox stress. In contrast, a broader set of proteins involved in intermediary metabolism, cell adhesion and ER stress were modified at high levels of ENP-induced redox stress. RNA silencing of glutaredoxin, a major enzyme that reverses SSG modifications, also markedly reduced macrophage bacterial phagocytic activity, confirming an important role of SSG in modifying innate immune function. Our results provide unique insights into the proteins that serve as sensitive ROS sensors and may facilitate cellular adaption to ENPs, versus targets of ENP-induced oxidative stress that are linked to irreversible cell outcomes.

1510 Method for Identification of Low Soluble, Biopersistent Dusts (GBS)

O. Creutzenberg, T. Hansen, S. Schuchardt, T. Tillmann and J. Knebel. *Fraunhofer ITEM, Hannover, Germany.* Sponsor: C. Dasenbrock.

Fine fractions of dusts are of high relevance for workplace safety due to their potential to induce lung diseases. Respirable biopersistent granular dusts (GBS) are defined as fine dusts with a negligible physiological solubility and no exhibition of a surface chemistry-related toxicity. The MAK Commission derived a new threshold value of 0.3 mg/m3 for GBS excluding any adverse effects on lungs upon chronic exposure to GBS (no volumetric lung overload). - The objectives of the project were to derive a general value for the term 'low soluble'. In addition, measuring the inflammatory response in lung lavage fluid, it was investigated whether besides microscaled also nano dusts could be included in the GBS class. - TiO2 (rutile, micro), TiO2 (anatase, nano), Eu2O3 (micro-nano mixed), BaSO4 (micro), ZrO2 (micro) and amorphous SiO2 (nano) were compared analysing the solubility in the lung fluid (day 3, 28 and 90) and the lung toxicity after intratracheal instillation in rats (day 3 and 28). Two volumetric doses of 0.5 and 1.5 µl per rat were administered to Wistar rats by intratracheal instillation; these volume doses resulted in a non-overload and moderate overload of lungs, respectively. - The differential cell count showed only slight inflammatory cell levels after treatment with TiO2 (rutile) and BaSO4. In contrast, the TiO2 (anatase) showed a stronger response. The rare earth Eu2O3 (micro-nano) dust showed the strongest effect including a red-coloured lung lavage fluid. ZrO2 and amorphous SiO2 showed a strong acute response after 3 days, however, mostly complete recovery after 28 days. These data were validated by using the physiological exposure path (subacute inhalation). GBS have to fulfill a 'low solubility' and a 'non-significant inflammatory response'. Most nanoscaled dusts will not meet these criteria and an individual toxicological characterization seems to be necessary for this particle class. The project was funded by the Federal Institute for Occupational Safety and Health (BAuA), Dortmund - F 2336.

1511 Surface and Size-Dependent Effects of Silver Nanoparticles on Two Aquatic Invertebrates: *D. magna* and *G. fossarum*

S. Cambier[3], K. Mehennaoui[3], E. J. Keuzenkamp[2], A. Georgantzopoulou[1,3], T. Serchi[3], L. Giamberini[4] and A. C. Gutleb[3]. *[1]Norsk Institutt for vannforskning, Oslo, Norway; [2]Division of Toxicology, Wageningen University, Wageningen, Netherlands; [3]Environmental Research and Innovation (ERIN) Department, Luxembourg Institute of Science and Technology, Esch sur Alzette, Luxembourg and [4]Laboratoire Interdisciplinaire des Environnements Continentaux, Université de Lorraine, Metz, France.*

Silver nanoparticles (Ag NPs) are used more and more in consumer products, ranging from textiles, cosmetics, food packaging to electronics. This increase in their use will finally lead to their release in the environment where they will inevitably end up in aquatic environments and have a potential impact on their ecosystem. However, the fate of the Ag NPs in aquatic ecosystems is still not fully understood as well as their impacts on aquatic living organisms. For this purpose, *Daphnia magna* and *Gammarus fossarum*, two model species for aquatic environments, were exposed to different citrate stabilised silver nanoparticles (ci-Ag NPs: 20, 40, and 80 nm), non-citrate stabilised silver nanoparticles (Ag NPs: 20 and 200 nm), and silver nitrate to evaluated their potential toxicity. In parallel to this toxicity assessment the fate of the Ag NPs in the media was investigated by using the nanoparticles tracking analysis (NTA) approach. In the exposure media the Ag NPs present different behaviour (agglomeration, dissolution etc.) depending on their sizes and surface chemistry. Indeed, both Ag NPs of 20 nm formed aggregates of 100 nm whereas Ag NPs of 40 and 80 nm were more stable. This study showed that ci-Ag NPs and non-stabilized Ag NPs are more toxic in acute conditions on D. magna in comparison to *G. fossarum*. Moreover, *D. magna* exposure revealed that ci-Ag NPs are more toxic than the non-stabilized one in a size and concentration manner. However, under chronic conditions a significant increase in mortality rates occurred when *D. magna* was exposed to Ag NPs of 200 nm at equitoxicity level (EC5 and EC1) compared with the other nanoparticles.

1512 Nanomaterials-Induced Alteration of Telomere Status: A Comparative Approach with *In Vitro* and *In Vivo*, the Nematode *Caenorhabditis elegans*

N. Chatterjee, Y.-H. Kim and J. Choi. *Environmental Engineering, University of Seoul, Seoul, Korea, Republic of.* Sponsor: B.-H. Lee.

The telomere, DNA-protein structures that cap the ends of chromosomes, dynamics and length are associated with genomics instability, cancer and various age related diseases. Evidences are rapidly increasing that telomere homeostasis might be affected by environmental and occupational exposures of chemicals, such as, air pollution (i.e, particulate matter (PM), benzene/ toluene, polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, pesticides, lead, chromium, arsenic etc). It is well established that the nano-biological interactions could possess a potential threat to environmental and human health due to the increasing use of naomaterials. The aim of the present study was to investigative the effect of engineered nanomaterials on telomere homeostasis in *in vitro* and *in vivo*, the nematode *Caenorhabditis elegans*, models. We screened various nanomaterials (metallic nanoparticles - AgNP, AuNP; carbon nanomaterials - MWCNTs, graphene nanos, oxide nanomaterials - TiO2, SiO2) with hTERT(+/-) knock out cell lines for *in vitro* detection while life span of the trt-1 (the telomerase mutant) and mrt-2 (possess shortening of telomere with each generations) mutants for *in vivo*. Among all the most sensitive one to cause decrease in life span of the telomere related mutants while SiO2, SLGO and MWCNT-OH were found to be sensitive in hTERT(+/-) knock out cell lines. Moreover, SiO2 and SLGO treatments displayed aging marker (lipofuschin accumulation in *C. elegnas*, cell senescence in cultured cells) in a dose dependent manner. Taken together, our results showed that nanomaterials could accelerate telomere shortening and in turn aging or age related diseases.

1513 Human RBC As a Surrogate to Study Particle-Induced Phagolysosomal Damage

T. Ziglari, D. Anderson, L. Fisch and A. Holian. *University of Montana, Missoula, MT.* Sponsor: A. Holian.

Recent years have witnessed unprecedented growth of research and applications in the area of nanoscience and nanotechnology. However, to leverage the benefits of nanotechnology in daily life, research is needed to understand health and safety issues associated with these materials. One of the most likely routes of exposure to particles is through inha-

lation. Although the role of lung cells in the defense or development of particle-induced diseases has been examined, the physicochemical properties of particles affecting the cellular responses and the overall mechanism of toxicity are not fully understood. Working with phagolysosome membranes is challenging therefore, an alternative model of membranes was explored. Therefore, we considered human red blood cells (RBC) as a surrogate for investigate membrane damage in two different media, phosphate buffered saline (PBS) and citrate buffered saline (CBS), and measured the hemolytic activity of crystalline silica compared to a series of nanoparticles (CeO2, TiO2, CuO, and ZnO). RBC hemolysis was measured using a 96-well plate format at 37 for 4 hrs with constant agitation. The pattern of hemolysis was different while using PBS versus CBS; when PBS was used, silica showed the highest hemolytic activity, then nickel and cerium, while other particles had lower hemolysis. The results are consistent with the known low activity of TiO2 nanospheres and the suspected dissolution of CuO and ZnO in phagolysosomes. But when citrate buffer was used, only silica had hemolytic activity. This suggests that citrate can cap the nanoparticles, but not affect silica. Finally, there was no indication of gross membrane disruption in the RBC, suggesting that the particles caused RBC hemolysis through the formation of small membrane pores. Further studies are necessary to understand the exact mechanism of interaction between particles and RBC membranes. This study was supported by NIH grants R25 ES022866-01, R01 ES023209 and P30 GM103338.

1514 Assessment of Bactericidal Effects of Food Contact Materials Containing Silver Nanoparticles and Expression of Silver Resistance Genes during Subchronic Exposure

L. Valencia, K. Williams, K. Gokulan, R. Trbojevich, C. Cerniglia, M. Boudreau and S. Khare. *Division of Biochemical Toxicology, National Center for Toxicological Research, US FDA, Jefferson, AR; Division of Microbiology, National Center for Toxicological Research, US FDA, Jefferson, AR.* Sponsor: R. Trbojevich.

Silver for centuries has been utilized as an antimicrobial against pathogenic bacteria. Manipulation of particles at a nano scale has led to the ingenuity of engineering nanoparticle embedded products known as Food Contact Materials (FCM). Silver nanoparticles (AgNP) are one of the widely used metal nanoparticles for FCM due to its antimicrobial properties. The antimicrobial and toxicological properties of AgNP are particle size, shape and dose -dependent. Like many antimicrobial agents, growing public health concerns is if the antimicrobial property of AgNP may also cause the antimicrobial resistance in microbes. It has been reported that some bacterial strains have the capability to become silver resistant when exposed to high concentrations of silver. The silver resistance is known to be plasmid mediated (pMG101) or in some particular cases also on bacterial chromosome. This study was initiated to determine if subchronic exposure of AgNP may promote silver resistance to the gut microbiome. In this study, antimicrobial activity was measured by identifying the reduction in bacterial bio-burden caused by the exposure to FCM (food storage containers, food wrapping material, and cutting board) that advertise antimicrobial activity. Furthermore, studies were conducted to assess if subchronic exposure of silver nanoparticles may induce the silver resistance in gut mucosa associated commensal bacteria. To achieve this, gut mucosa DNA obtained from male and female Sprague-Dawley rats that were orally gavaged to three sizes and three doses of AgNP for 13 weeks was used to analyze the expression of silver resistance genes. Our findings from FCM trials suggest very limited capacity for the bactericidal effect against tested bacterial species. Preliminary results reveal silver resistance genes (SilE and SilS) were detected in both male and female; however, higher expression of Sil P was predominantly found in male rats. Public Health Concern: If consumer exposure to FCM products containing silver nanoparticles alters the intestinal microbiome and gastrointestinal tract functions.

1515 Alteration of Allergic Response Following an Acute Pulmonary Exposure to Nickel Oxide Nanoparticles in a Murine OVA Asthma Model

K. A. Roach[2], C. E. McLoughlin[1], S. E. Anderson[1,2], A. B. Stefaniak[1], D. Schwegler-Berry[1] and J. R. Roberts[1,2]. *[1]CDC/NIOSH, Morgantown, WV and [2]West Virginia University, Morgantown, WV.*

Nickel is a causative agent of both dermal and respiratory allergy; however, despite the emerging nanotoxicological concept of size-dependent particle toxicity and increases in industrial use, nickel nanoparticles' effect on the immune system compared to larger nickel particles has not been explored. This study aimed to assess alterations in allergic disease following an acute exposure to NiO nanoparticles in an ovalbumin (OVA) allergy model, as compared to larger-sized NiO particles. Prior to *in vivo* exposures, two different forms of NiO particles were characterized in dispersion medium (DM: vehicle control). NiO particles had a primary size of < 5 µm diameter and served as a nickel control (NiO) and nano-sized NiO particles had a primary particle size ~50 nm diameter (NiON). On day 0, mice were oropharyngeally aspirated with 40 µg of NiO, NiON, or DM. On days 1 and 10, mice were sensitized to OVA via intraperitoneal injection and were challenged with OVA on days 19 and 28 via oropharyngeal aspiration. A separate group of particle-treated mice were not exposed to OVA and served as the non-allergic control groups. Following OVA-challenge on day 28, airway hyperreactivity (AHR) was assessed. On day 29, lungs were lavaged, IgE, cytokines, and lactate dehydrogenase activity were assessed in the fluid, and lavage cells were phenotyped. Serum and lung-associated lymph node cells were also collected for analysis. At day 29, there were no significant increases in lung injury or inflammation in mice exposed to NiO or NiON in the absence of OVA-induced allergy. OVA-sensitized animals treated with NiO did not exhibit any significant differences in allergic parameters when compared to OVA controls. However, AHR, lung-associated lymphocytes, and lung eosinophils were significantly increased in OVA-exposed animals treated with NiON when compared to the OVA control and the OVA-exposed NiO group. Overall, NiON, but not NiO, significantly altered the severity of allergic response in the OVA model following an acute exposure to particles prior to sensitization. These results suggest that exposure to a given concentration of nano-sized NiO may pose a greater health risk in populations susceptible to pulmonary allergic disease than that of its larger counterpart.

1516 Ingestion of Nanoparticles Used in Food Interrupts Intestinal Epithelial Function and Triggers Pro-Inflammatory Signaling

Z. Guo[2], E. Tako[1] and G. Mahler[2]. *[1]Department of Food Science, Cornell University, Ithaca, NY and [2]Department of Biomedical Engineering, Binghamton University, Binghamton, NY.*

Ingestion of nanoparticles (NPs), mainly as TiO2 and SiO2, from products such as agricultural chemicals, processed food, and nutritional supplements is nearly unavoidable. Our previous work found polymeric NPs affected iron absorption, and our current goal is to use an *in vitro* cell culture model of the gastrointestinal tract to examine how NPs widely used in food affect mineral absorption. An *in vitro* cell culture model composed of Caco-2 and HT29-MTX cells was exposed to physiologically relevant doses of 30 nm TiO2 or SiO2 NPs for acute (4 hours) or chronic (5 days) time periods. Doses of SiO2 and TiO2 significantly altered Zn and Fe transport. The transepithelial resistance (TER) and occuldin expression remained statistically the same between controls and acutely exposed cultures, meaning that changes in nutrient transport were not due to alterations in tight junction functionality. Changes in nutrient transport could be related to significantly upregulated production of reactive oxygen species following NP exposure. Chronic exposure also significantly down regulated genes expression of Fe and Zn transporters (DMT1, DCYTB, FPN1, HEPH, ZnT1, ZIP1), and interrupted protein synthesis of SGLT1, FABP2, and FABP. Pro-inflammatory signaling was also significantly upregulated. TNFα gene expression was upregulated (~20-30 fold) in response to acute doses of SiO2 or TiO2. SiO2 increased NFκB1 gene expression more than two-fold. IL8 gene expression was ~2-4 fold higher following chronic NP exposure. Overall, iron and zinc transport mechanisms in intestinal epithelial cells are disturbed ingestion of physiologically relevant doses of NPs.

1517 Dermal Application of Nanoporous Silica Nanoparticles Aggravates Atopic Dermatitis

S. Lee[2], J. K. Choi[1] and S.-H. Kim[1]. *[1]Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu, Korea, Republic of and [2]Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup-si, Jeonbuk, Korea, Republic of.*

Nanoporous silica nanoparticles (NSN) have attracted considerable attention as a molecular sieve due to their inherent properties, such as a large pore volume and surface area, as well as a tunable pore size. However, due to the known properties of nanoparticles (NPs) as causative agents affecting skin barrier penetration and immunologic responses, understanding dermal penetration, as well as possible toxicity of NSN is important issue. The aim of our study was to define the effects of pore structural conditions of silica NPs on atopic dermatitis (AD). We synthesized NSN and colloidal (non-porous) silica NPs (CSN) with only difference of pore architectures, such as specific surface area and pore volume. AD was induced on the ears of BALB/c mice using house dust mite extract (*Dermatophagoides farinae* extract, DFE). NSN and CSN were topically applied to AD mice (100 and 500 µg/ear) over a three week period. While CSN-treated mice showed no difference in the severity of AD induced by DFE, NSN-treated counterparts exhibited exacerbated

AD symptoms when compared to untreated control mice. As results, NSN showed much higher increase of ear and epidermal thickness, infiltration of immune cells (eosinophils, mast cells) and proliferation of immune cell than CSN. NSN led to significant increase of AD specific immunoglobulins and histamine in serum, proliferation of T cells in lymph node, and expression of Th1/Th2/Th17 cytokines in ear tissue. Interestingly, NSN-induced AD symptoms were significantly reduced by oil treatment as a moisturizer. To define the underlying mechanisms of action, human keratinocytes (HaCaT) model was used. Our analyses revealed that NSN treatment elicited an increase in pro-inflammatory cytokines and chemokines via activation of nuclear factor-κB pathway. Taken together, these results indicate that *in vivo* exposure to NSN caused more damage to skin inflammation than that of CSN because of their hydroscopic properties, and provide useful information for the continued development of silica NPs for dermal application.

PS 1518 Proteomic Analysis of Protein Carbonylation: a Useful Tool to Unravel Nanoparticle Toxicity Mechanisms

M. D. Driessen[2], S. Mues[1], A. Vennemann[3], B. Hellack[4], A. Bannuscher[2], V. Vimalakanthan[2,5], C. Riebeling[2], R. Ossig[1], M. Wiemann[3], J. Schneckenburger[1], T. A. Kuhlbusch[4], B. Renard[5], A. Luch[2] and A. Haase[2]. *[1]Biomedical Technology Center, Westfälische Wilhelms-Universität, Münster, Germany; [2]German Federal Institute for Risk Assessment, Berlin, Germany; [3]IBE R&d gGmbH, Münster, Germany; [4]Institute of Energy and Environmental Technology (IUTA) e.V., Duisburg, Germany and [5]Robert-Koch-Institut (RKI), Berlin, Germany.*

Oxidative stress is considered to be a major paradigm to explain NP toxicity. Here we focused on protein carbonylation as a consequence of oxidative stress for a set of 24 different nanoparticles, used as either plane ("pristine") materials or with different surface coatings. In parallel several *in vitro* and *in vivo* toxicity endpoints have been analyzed. We used well characterized NP of 10 nm (ZrO2), 15 nm (SiO2) and 50 or 200 nm (Ag), furnished either with acidic, basic or polymeric functionalities. In addition TiO2 (NM-103,-104,-105), ZnO (NM-110, -111), CNT (NM-400, -401, -402), SiO2 (NM-200, -203), Ag (NM-300K), BaSO4 (NM-220) and AlOOH were analyzed. In a screening approach we studied time- and dose-dependent carbonylation of all NP in NRK-52E cells via 1D immunoblots. Data were correlated with cytotoxicity (WST-8, LDH assay), ROS formation (DCFDA assay) and surface reactivity (ESR spectroscopy). We applied a 2D-MALDI-MS/MS proteomics approach to identify the modified proteins. For selected NP we also analyzed lung tissues homogenates after intratracheal instillation into rat lungs. Eleven out of 24 NP induced protein carbonylation in NRK-52E cells. The degree of protein carbonylation correlated well with overall *in vitro* toxicity. The 2D approach revealed a complex and distinct pattern of carbonyls. Modified proteins were identified as proteins of cytoskeleton, HSP, or proteins of major cellular pathways (i.e. glycolysis). Carbonyl modifications occurred also in lung tissue homogenates, indicating the relevance of *in vitro* findings. Analysis of poteomics data with hierachical cluster analysis and principal component analysis revealed differential results for the NM with respect to induced carbonyl patterns. Taken together, analysis of protein carbonylation appears useful to describe toxic effects of NP and to better understand underlying molecular mechanisms of toxicity. Furthermore, proteomics data can support NM classification.

PS 1519 Toxicological Effects of a Food Additive Tio2 to the Nematode *C. elegans*: Does the Nano Matter?

H. Ma[1], C. Yuan[1], X. Gao[1], S. Li[2] and L. Wallis[3]. *[1]University of Wisconsin Milwaukee, Milwaukee, WI; [2]US EPA/Mid-Continent Ecology Division, Duluth, MN and [3]Western Washington University, Bellingham, WA.*

Titanium dioxide (TiO2) has been used as a food additive for years and bulk TiO2 (in micro size) is generally considered safe for human and ecosystem health because of its inertness. Recent studies have found that significant portion of those bulk TiO2 fall in the nano range (i.e., < 100 nm) and those nano-sized particles may pose unforeseen health risks for human and ecological species. The present study investigated the toxicological effects of a commercially available food additive TiO2 as compared to the industrial materials nano TiO2 (P25) and bulk TiO2 in a model organism the nematode *C. elegans*. Particle characterization including size distribution and crystal structure were performed using scanning electron microscopy (SEM) and X-ray diffraction (XRD). Photoreactivity of the TiO2 materials was determined using a fluorescence based assay as well as methylene blue degradation. Toxicological endpoints included lethality, reproduction, lifespan, and transgene expression, under both UV irradiation and regular laboratory lighting. Uptake and biological distribution of the TiO2 particles within in the worms were examined using SEM coupled with energy dispersive X-ray spectroscopy (EDS). Our results indicate that approximately 20% of the food additive TiO2 has particle size below 100 nm. Under environmentally relevant UV radiation, the P25 showed greatest photoreactivity and photoinduced toxicity, followed by the food additive TiO2, and lastly the bulk TiO2. Translocation of the daf-16::GFP expression from cytoplasm to nucleus within the test animals indicates that oxidative stress is involved in the observed toxicological effects. Toxicological effects under laboratory lighting was less significant with only P25 showing effects on reproduction. Uptake of TiO2 into worm intestinal system was obvious for all three TiO2 materials, however, uptake into other organs was most significant for P25, followed by food additive TiO2, and no significant uptake of bulk TiO2 was observed. Our findings suggest that food additive TiO2 containing nano-sized particles induces greater toxicological effects than micro-sized bulk materials. Given the widespread use of food additive TiO2 and the related human exposure, more extensive studies are needed to better understand their potential health implications to humans.

PS 1520 Indirect and Direct Nanomaterial Interaction Influences Vascular Reactivity

V. C. Minarchick[3], P. A. Stapleton[2], J. H. Shannahan[3], E. M. Sabolsky[2], T. R. Nurkiewicz[2] and J. M. Brown[3]. *[1]Center for Cardiovascular and Respiratory Sciences, West Virginia University, Morgantown, WV; [2]Mechanical Engineering, West Virginia University, Morgantown, WV and [3]Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Cerium dioxide nanoparticles (CeO2 NP) have been shown to cause vascular dysfunction. However, it is unknown if this dysfunction is due to circulating factors and/or direct nanomaterial interaction. We hypothesized that the vascular dysfunction is due to endothelial changes, which may interrupt cellular signaling, and these alterations (i.e. oxidative stress, inflammatory markers) will be dependent on the physicochemical properties of the nanomaterial. Rat aortic endothelial cells (RAEC) were exposed to CeO2 NP (0, 6.25, 12.5, 25, and 100 µg/ml) for 24 hours. Cytotoxicity, alterations in inflammatory and surface adhesion markers (IL-6, VCAM-1, and ICAM-1 via mRNA expression and western blot analysis), nitric oxide synthase (NOS) expression, and reactive oxygen species (ROS) generation (via mitochondrial superoxide release and cellular hydrogen peroxide production) were assessed following exposure. Additionally, isolated naïve arterioles were intraluminally exposed to CeO2 NP (0, 6.25, 12.5, 25, and 100 µg/ml) or the supernatant from exposed RAEC. Endothelium-dependent and -independent reactivity was assessed with acetylcholine (ACh, 10-9-10-4 M), and sodium nitroprusside (SNP, 10-9-10-4 M). Finally, enhanced dark field microscopy was used to determine CeO2 NP cellular uptake. Following exposure, RAEC had no change in cellular viability and NOS expression. There was a significant increase in IL-6 production and approximately a 20% decrease in ROS generation at the lowest concentration. Furthermore, CeO2 NP exposure significantly impaired ACh (75±8% vs. naïve control) and SNP (58±5% vs. naïve control) induced dilation. Taken together, these results indicate that both direct and indirect CeO2 NP interactions likely alter cell-cell and/or intracellular signaling within the vascular wall. R01-ES019311 (JMB), R01-ES015022 (TRN), K99-ES024783 (PAS), K99-ES024392 (JHS)

PS 1521 The Effect of Acute Exposure to TiO2 Nano Fiber in the Lungs of Sprague Dawley Male Rats

D. A. Hunter, C. A. Mays, D. Patel, J. Wu and W. E. Gato. *Chemistry, Georgia Southern University, Statesboro, GA.*

In the past two decades, nanotechnology has grown tremendously. Synthetic nanomaterials have many unique chemical and physical properties, mainly due to their huge specific surface area and quantum confinement effect. Specifically titanium dioxide (TiO2) nanomaterial have high stability, anticorrosive and photocatalytic properties. These nanomaterials have applications for semiconductor photocatalysis, treatment of water or as a photoactive material in nanocrystalline solar cells. Recent studies show that TiO2 nanoparticle exposed to UV light irradiation show antibacterial properties and because of this they are under investigation for its use in nanomedicine. However, there are concerns over adverse effects resulting from bio-effects. The objective of the present study is to investigate the adverse effect associated with acute ingestion of TiO2 nanofiber (TDNF). TDNF was fabricated via electrospinning method, followed by dissolution in water. Six to seven weeks male Sprague Dawley rats were exposed to a total of 0, 40 and 60 ppm of TDNF for two weeks via oral gavage. Rats were euthanized via CO2 asphyxiation. Various organs including the lung was snap-frozen in liquid nitrogen. Sections of the tissues were also fixed in neutral

buffered formalin for histopathological analysis. Preliminary morphometric data indicate slight reduction in body and lung weight. Measure of total protein also decreased in rats that ingested TDNF. Anatomical assessment of the pulmone indicate collapsed lung, bronchiolar arteriole hyperplasia, mild bronchiolar and alveolar hemorrhage, perivascular edema. The study is still ongoing to investigate genome-wide expression of transcripts in the lung.

1522 Positively Charged Nanoparticles Lead to Artifacts in a *C. elegans* Toxicity Assay

S. K. Hanna, A. Montoro Bustos, M. R. Winchester, B. C. Nelson, J. T. Elliott and E. J. Petersen. *Materials Measurement Lab, National Institute of Standards and Technology, Gaithersburg, MD.* Sponsor: E. Coskun.

The unique properties of engineered nanoparticles (ENPs) may lead to unforeseen interactions and artifacts in toxicity assays that were originally designed for soluble chemicals. Yet, there is a lack of test methods specifically tailored to ENPs, which has hindered efforts to understand the potential environmental consequences of ENP release. Here, we detail our efforts to adapt a *C. elegans* toxicity assay for use with ENPs, including a cause-and-effect analysis to identify potential sources of error followed by a sensitivity analysis to quantify the error. We found that shaking plates during the assay decreased growth by as much as 36% and changing the concentration of feed in the assay greatly altered the toxicity of the positive control, benzylcetyldimethylammonium chloride (BAC-C16). We also found that three commonly used *C. elegans* media formulations produced similar results in the toxicity assay, allowing for flexibility to suit ENP characteristics and reduce agglomeration. Additionally, in our testing we discovered that positively charged polystyrene nanoparticles (PSNPs) elicit toxic effects on *C. elegans*. However, those impacts were due to an interaction with *E. coli* that is used as feed in the assay and impacts on growth were more variable for PSNPs (52%) compared to BAC-C16 (9%). *E. coli* and positively charged PSNPs formed heteroagglomerates, leading to large particles that the nematodes cannot ingest. This, in turn, reduces growth and reproduction. We repeated this test using Au ENPs with coatings ranging from positive to negative and found similar results with our positively charged Au ENPs compared to PSNPs. In contrast, analysis of nine other Au nanoparticles that were neutrally or negatively charged did not show particle heteroagglomeration nor toxicity to *C. elegans*. We were able to quantify the *E. coli* aggregation via turbidity measurements and present an alternative assay, which does not include bacteria, to accommodate positively charge ENPs.

1523 Sphingosine Kinase 1 Deficiency Protects Against Particle-induced Lysosome Membrane Permeabilization and NLRP3 Inflammasome Activity in Macrophages

F. Jessop, R. F. Hamilton and A. Holian. *Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT.*

Lysosome membrane permeabilization precedes activation of the NLRP3 Inflammasome and release of associated inflammatory cytokines including IL-1β. Particle exposure is known to induce lysosome membrane permeabilization in macrophages through mechanisms that have not yet been elucidated. Sphingosine kinases have been implicated in lysosome destabilization during apoptosis, but their role following particle exposure is not known. In this study, the contribution of sphingosine kinase 1 to particle-induced lysosome membrane permeabilization was addressed in bone marrow derived macrophages from WT and sphingosine kinase 1 KO mice. Measuring cathepsin and N-acetyl-beta-D-glucosaminidase activity in the cytosolic fraction, following selective digitonin extraction, was used to assess lysosome membrane permeabilization after exposure to multiple types of particles including: titanium nanobelts, titanium nanospheres, nickel oxide nanospheres, multi-walled carbon nanotubes, crocidolite asbestos, and crystalline silica. Sphingosine kinase 1 KO macrophages had significantly less cytosolic cathepsin and N-acetyl-beta-D-glucosaminidase activity than WT with most particle exposures. IL-1β production and cytotoxicity (LDH) were also less in sphingosine kinase 1 KO macrophages 24 hours after particle exposure. Addition of the inhibitor SPHK I2, which targets all sphingosine kinases, further decreased cytokine production from sphingosine kinase 1 KO macrophages, suggesting partial compensation by sphingosine kinase 2. Together, these results show a partial role for sphingosine kinases in mechanisms responsible for particle-induced lysosome membrane permeabilization.

1524 Examining the Hepatic Effect of TiO2 Nano Fiber Ingestion in Sprague Dawley Rats

K. B. Anderson, D. A. Hunter, C. A. Mays, D. Patel, J. Wu and W. E. Gato. *Chemistry, Georgia Southern University, Statesboro, GA.*

A nanomaterial refers to a material having structures in the nanometer size range. These materials differ from bulk materials in the way in which they behave. That is they exhibit a different scaling property and quantum effects thus affecting the chemical reactivity of materials including their mechanical, optical, electric, and magnetic properties. As a consequence nanomaterials have applications in electronics, transportation and telecommunication, imaging, biomedical applications, pollution remediation, cosmetics, coatings, as insulation materials and as well as nanocomposites. Specifically, titanium dioxide nanomaterials has been widely employed in pigments, sunscrees, pains, ointments, toothpaste and photocatalytic splitting of water. The goal of the present study is to examine hepatic effects associated with the ingestion of TiO2 nanofiber (TDNF). TDNF was fabricated via electrospinning method, followed by dissolution in water through the agitation. Six to seven weeks old male Sprague Dawley rats ingested 0, 10, 15 ppm twice a week for a total of 0 ppm, 40, 60 ppm TDNF for the duration of the study. At the end of the treatment period, animals were euthanized via CO2 asphyxiation. Blood was drawn via cardiac puncture. The liver and other organs were autopsied. Some of the tissues were fixed in neutral buffered saline and the others frozen in liquid nitrogen until analysis. Clinical chemistry assessment of alanine transferase and total protein will be carried out. Histopathological analysis of the liver showed no significant difference between groups, though moderate glycogen accumulation were noted in the exposed animals. Serum total protein levels were dose-dependently reduced in treated groups. Additional study will involve proteomic assessment of bulk proteins will be performed to understand and identify health impact associated with exposure to TDNF.

1525 Stable Isotope Method to Measure Drug Release From Nanomedicines

S. T. Stern, S. Skoczen and S. E. McNeil. *Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Frederick National Laboratory, Frederick, MD.*

Existing methods to measure nanomedicine drug release in biological matrices are inadequate. A novel drug release method utilizing stable isotope dilution has been developed. Stable isotope-labeled drug is spiked into plasma containing nanomedicine. The labeled drug equilibrates with plasma components identical to the normoisotopic drug released from the nanomedicine formulation. Therefore, the ultrafilterable fraction of the isotope-labeled drug represents a reliable measure of free normoisotopic drug fraction in plasma, and can be used to calculate nanomedicine encapsulated and unencapsulated drug fractions. To demonstrate the utility of this method, we performed a plasma drug release study with both a fast releasing commercial docetaxel formulation, Taxotere®, and a delayed releasing nanomicellar formulation of a docetaxel prodrug, Procet 8. The instability of the unencapsulated prodrug in plasma allowed us to compare our calculated prodrug release and docetaxel conversion with the actual docetaxel concentration measured directly without fractionation. Drug release estimates for the fast releasing Taxotere formulation demonstrated accuracy deviation and precision (%CV) of <15%. For the controlled release Procet 8 formulation, we calculated a slow release and conversion of the prodrug in rat plasma that was highly correlated with the direct docetaxel measurement (R2=0.98). We believe this method will have tremendous utility in development and regulatory evaluation of nanomedicines, and aid in determination of generic bioequivalence.

1526 Computational Dosimetry Reveals the of Role Particles and Ions in the Toxicity of Soluble Silver Nanoparticles

D. G. Thomas[2], J. Smith[2], V. K. Kodali[1], M. Littke[2], H. Jolley[2], B. D. Thrall[2], J. G. Pounds[2] and J. G. Teeguarden[2]. [1]*National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV and* [2]*Pacific Northwest National Laboratory (PNNL), Richland, WA.*

When suspended in cell culture media, nanomaterials composed of soluble metals such as silver can undergo dissolution resulting in ion formation and altered particle properties (e.g. mass, morphology, etc.) ultimately modulating cellular dose. Cultured cells are exposed not just to particles, but to a complex, dynamic mixture of particles, free ions, and ion-ligand complexes. Through a variety of nanoparticle dissolution, cellular uptake, and nanoparticle property experiments, a computational model (*In Vitro* Sedimentation, Diffusion, Dissolution, and

Dosimetry Model (ISD3)) was developed to predict particle and ionic dosimetry to cells exposed to silver nanoparticles using a population balance approach. Using three different cell types (bone marrow derived macrophages from wild-type C57BL/6J mice and Scavenger Receptor A deficient mice and RAW 264.7 macrophages), ISD3 accurately predicts nanoparticle dissolution in cell culture media and silver dosimetry for two sizes of silver nanoparticles (20 and 110 nm) and silver ions. Toxicity to those three cell types were measured after exposure to different concentrations of two sizes of silver nanoparticles, and ISD3 was used to predict dosimetrics of nanoparticle exposure conditions. Multiple mixed linear regression model analyses suggest that intracellular nanoparticle surface area was the best predictor of cytotoxicity and viability of all experimental conditions (e.g. cell type, nanoparticle size, etc.). Further experimental results demonstrate that ions formed from nanoparticle dissolution may rapidly complex with ligands reducing toxicity. Overall these results suggest intracellular nanoparticle surface area is the most important determinate of silver nanoparticle toxicity, implicating intracellular dissolution as a potential mechanism. Supported by National Institute of Environmental Health Sciences (NIEHS) U19 ES019544.

1527 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Low Level Cadmium Exposure

O. O. Ogunrinola[4], A. D. Wusu[4], O. K. Afolabi[3], E. O. Abam[1], E. A. Balogun[5], O. O. Odukoya[2] and O. Ademuyiwa[2]. *[1]Biochemistry, Bells University of Technology, Ota, Ogun-State, Nigeria, Ota, Nigeria; [2]Biochemistry, Federal University of Agriculture, Abeokuta, Ogun-State, Nigeria, Abeokuta, Nigeria; [3]Biochemistry, Ladoke Akintola University, Ogbomosho, Oyo-State, Nigeria, Ogbomosho, Nigeria; [4]Biochemistry, Lagos State University, Ojo-Lagos, Nigeria, Lagos, Nigeria and [5]Biochemistry, University of Ilorin, Ilorin, Kwara-State, Nigeria, Ilorin, Nigeria; [6]Chemistry, Federal University of Agriculture, Abeokuta, Ogun-State, Nigeria, Abeokuta, Nigeria.*

Exposure to cadmium induces different biochemical responses. In order to investigate the effects of low level cadmium exposure on 3-hydroxy-3-methylglutaryl CoA(HMG-CoA)reductase in the liver, brain and plasma lipid metabolism spectrophotometrically, rats (n = 24; mean weight = 250 g) were exposed to cadmium chloride (100, 200 and 300 ppm) in their drinking water for 12 weeks, while control animals (n = 8) received distilled water for the same period. Exposure to cadmium resulted in highest accumulation of cadmium in the liver from 6.14±0.22 µg Cd/g tissue in control to 54.87±4.86 µg Cd/g tissue in 300ppm dose. Hypocholesterolemia characterized the effect of cadmium with a 50% reduction of control. In the organs examined, highest cadmium dose produced a significant dose-dependent ($p<0.05$) decrease in hepatic cholesterol (45%) and hepatic microsomal cholesterol (42%) concentrations compared to control. There was a non-dose-dependent decrease of 65% in brain cholesterol concentration and dose-dependent of 40% reduction in the brain microsomes of rats exposed to cadmium. Hepatic HMG-CoA reductase activity was reduced by 55% and that of the brain by 39%. Positive associations were observed between hepatic cadmium and HMG-CoA reductase ($r=0.675, p<0.01$); brain cadmium and brain HMG-CoA reductase ($r=0.636, p<0.01$). Spectrophotometric assay of this study revealed that cadmium-induced inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity which indicates that the down-regulation could be responsible for its atherogenic effects.

1528 Involvement of Polyubiquitin-Coding Gene, UBB, in Cadmium Toxicity in Human Proximal Tubular Cells

J.-Y. Lee, M. Tokumoto and M. Satoh. *School of Pharmacy, Aichi Gakuin University, Nagoya, Japan.*

Cadmium (Cd) is an environmental contaminant that induces severe clinical symptoms in various tissues including the kidney. Overaccumulated Cd in the kidney damages the proximal tubules. Our previous study demonstrated Cd-induced suppression of the UBE2D gene family, one of the ubiquitin-conjugating enzyme families1). However, the precise role of ubiquitin-coding genes in Cd toxicity remains to be understood. In this study, we investigated the effect of Cd on expression of the ubiquitin-coding genes UBB, UBC, UBA80, and UBA52 in HK-2 human proximal tubular cells. Using MTT assay, it was demonstrated that HK-2 cells treated with 40 µM Cd for 6 hr exhibited 50% cell viability, whereas a 3 h treatment did not induce cytotoxicity. Therefore, we investigated the effect of Cd on expression of 4 Ub-coding genes (UBB, UBC, UBA80, UBA52) with 40 µM Cd for 1, 3, and 6 h by real-time RT-PCR. The 3 h treatment induced expression of UBB, UBC, and UBA80 with the exception of UBA52 compared with non-treated cells. Next, we employed siRNA transfection to target each Ub-coding gene to investigate whether expression changes in Ub-coding genes may affect the sensitivity of HK-2 cells to Cd. Transfection of UBB siRNA markedly decreased UBB mRNA level. Interestingly, UBB siRNA transfection decreased the sensitivity of HK-2 cells to Cd. Transfection of UBA80 siRNA markedly decreased UBA80 mRNA level. However, in contrast to UBB siRNA treatment, UBA80 siRNA-transfected cells exhibited the same Cd sensitivity as control siRNA. Because UBB disruption eliminated Cd toxicity in HK-2 cells, effect of UBB siRNA-treatment on the elevation of ubiquitinated proteins by Cd was examined using western blot analysis. Although the level of ubiquitinated proteins following UBB siRNA treatment alone was the same as for control siRNA treatments, Cd-induced protein ubiquitination in control siRNA treated cells was eliminated by UBB siRNA treatment. These results suggest that UBB is involved in Cd-induced increase of protein ubiquitination, and that accumulation of ubiquitinated proteins through increased UBB expression may contribute to Cd toxicity in HK-2 cells. [This work was partly supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan]

1) Tokumoto, et al., (2011) J. Toxicol. Sci., 36, 191-200.

1529 Low Dose Oral Cadmium Increases Airway Reactivity and Lung Neuronal Gene Expression in Mice

J. D. Chandler, C. Wongtrakool, S. A. Banton, S. Li, M. Orr, D. B. Barr, D. Neujahr, R. L. Sutliff, Y.-M. Go and D. P. Jones. *Emory University, Atlanta, GA.*

Inhalation of cadmium (Cd) from occupational exposures or smoking is associated with multiple lung diseases, but less is known concerning pulmonary effects of chronically ingested Cd at levels found in the human diet. Chronic exposure to low doses of Cd, which has a decades-long biological half-life in humans, leads to significant bioaccumulation with unclear toxicological ramifications, particularly for the lung. We exposed mice to 10 mg/L CdCl2 in drinking water for 20 weeks, causing a significant increase in lung Cd burden similar to that of non-occupationally exposed, non-smoking adult humans. Cd-treated mice had significantly increased airway hyperresponsiveness to methacholine challenge compared to controls ($p<0.05$). Using a MoGene ST 2.0 Exon gene array (Affymetrix), we observed that Cd significantly altered 443 genes at $p<0.05$. However, Cd did not elicit increased metallothionein transcripts as expected, a result confirmed by qRT-PCR. To identify the pathways most affected by Cd, transcripts were ranked by abundance and significance and uploaded to the Gene Set Enrichment Analysis applet (Broad Institute) for pathway enrichment. Neuronal receptors were the major inducible targets of Cd, enriching olfactory, cholinergic and serotonergic gene sets at FDR<0.05. Olfactory receptor (Olfr) transcripts, which control chemosensory pathways and airway hyperresposiveness, were the most enriched, and the 5 most significant genes (Olfr 97, 317, 341, 458 and 1416) were validated using qRT-PCR. Furthermore, targeted metabolomics revealed that metabolic precursors of neurotransmitters for nicotinic (choline), glutamatergic (glutamate) and serotonergic (tryptophan) receptors were significantly increased by Cd in lung tissue ($p<0.05$). In conclusion, lung burden from low dose oral Cd exposure was comparable to that of human adults and associated with increased bronchiolar reactivity through neuronal pathways. These results suggest more detailed investigations are needed to elucidate the potential health impacts of dietary Cd, especially on airway hypersensitivity and asthma risk.

1530 Low Level Cadmium Causes Dysglycemia and Decreased Serum Leptin in the db/db Mouse, a Model of Type II Diabetes Mellitus

J. Edwards. *Midwestern University, Downers Grove, IL.*

Diabetes is a growing worldwide epidemic. Cadmium (Cd) is a ubiquitous environmental pollutant that is associated with hyperglycemia and diabetes. In a recent publication, I show that after 12 weeks of Cd exposure in rats there is an increase in serum levels of glucose-dependent insulinotropic polypeptide (GIP) and a decrease in the level of the satiety hormone, leptin. Based on these observations I conducted a pilot study using db/db mice (type II diabetic mouse model) and lean mice. All animals were given daily subcutaneous injections of Cd at a dose of 0.6 mg/kg/day for two weeks then given no Cd for another two week period. At the end of the study, all animals underwent an oral glucose tolerance test (OGTT), days later serum samples were collected from 5 hour fasted animals. In the OGTT, Cd had the greatest effect on db/db mice, with all animals except one having blood glucose levels of 600 mg/dl or higher 30 min following an oral dose of glucose (2g/kg). At the same 30 min time point, the non-Cd treated db/db mice had an average blood glucose value of 507 ± 19 mg/dl. Db/db mice which have a point mutation in the leptin receptor had a fasting serum leptin value of 271.7 ± 28 (ng/ml) in control vs 170.2 ± 39 in Cd treated animals; $p \leq 0.05$. No significant

differences were detected in lean control (saline) mice vs Cd; 1.9 ± 0.6 vs 2.2 ± 0.9. Serum levels of GIP showed no statistically significant change in db/db mice however a trend was noticed with control db/db mice with 20.2 ± 5.6 (pg/ml) vs 11.9 ± 7.1 in Cd treated animals. Levels of GIP from lean mice also did not change with treatment with 4.8 ± 2.4 vs 2.1 ± 0.08 for control and Cd treatment, respectively. These results indicate that alterations in serum leptin may be a vital step in the etiology of Cd-induced dysglycemia.

1531 Involvement of Hyperglycemia or Cadmium Exposure in the Induction of the Polyol Pathway and Morphological Changes in Human Proximal Tubule Cells

B. A. Davis, S. H. Garrett, D. A. Sens and S. Somji. *Pathology, University of North Dakota, Grand Forks, ND.*

Diabetic nephropathy is a major cause of end-stage renal disease (ESRD), where prolonged exposure to hyperglycemia induces damage to proximal tubule cells of the kidney. Since progression to ESRD correlates to pathological changes in the tubular segments of the kidney, the effects of hyperglycemia in the PT portion of the nephron may be particularly relevant to the progression of DN. Development of this disease also is likely to occur in the context of exposure to other renal toxins, and the heavy metal cadmium may be the most relevant due to the accumulation of this metal in the major cell type involved in glucose reabsorption; proximal tubule cells. Preliminary microarray analysis has shown human proximal tubule cells exposed acutely and chronically to cadmium have an increased expression of an aldose reductase (AR) isoform, AKR1B10. This isoform along with AKR1B1 and sorbitol dehydrogenase (SORD) are involved in glucose metabolism under hyperglycemic conditions via the polyol pathway. The goal of this study was to verify and extend these observations in culture of human proximal tubule cells. For this purpose, HPT cells were exposed to one of the three following treatments; 5.5 (control), 7.5, 11, or 16 mM glucose concentrations for 8 days, 9, 27, 45 µM cadmium for 24 hours (acute), or 4.5, 9, 27 µM cadmium for 13 days (chronic). Real-time PCR was used to measure the expression level of these enzymes. Exposures to either hyperglycemia or cadmium stimulated a significant induction of AKR1B10 in proximal tubule cells; however, exposure to these renal toxins had no effect on AKR1B1 or SORD expression. We also observed glucose-induced loss of epithelial morphology that correlated to an induction of N- Cadherin (CDH2), a mesenchymal marker. These results are suggestive of potential synergistic effects of cadmium and hyperglycemia in the toxic responses of the proximal tubule during the development of diabetic nephropathy.

1532 The Inhibition of Transcriptional Activities of YY1 and FOXF1 Triggers the Expression of Renal Toxicity by Cadmium

M. Tokumoto, J.-Y. Lee and M. Satoh. *School of Pharmacy, Aichi Gakuin University, Nagoya, Japan.*

Cadmium (Cd) is a harmful heavy metal that induces renal toxicity, but this mechanism is not revealed. Our recent study demonstrated that Cd induced p53-related apoptosis via repression of UBE2D family (UBE2D1, 2, 3 and 4) gene expressions in rat proximal tubular cells (NRK-52E cells). However, it is unclear that why Cd repressed the expression of UBE2D family genes. The aim of this study is to clarify the involvement of the inhibition of transcriptional activity in the Cd-induced renal toxicity through the suppression of UBE2D family gene expression. Using the protein/DNA binding assay, it was shown that Cd decreased the activities of 15 transcription factors by less than 0.5-fold among the 65 transcription factors monitored. Yin-Yang-1 (YY1) is the transcription factor whose activity was inhibited the most. The core binding sequence of YY1 (GCNGCCATC) is high-accuracy with upstream sequences of UBE2D2 and UBE2D4 gene regions. Then, it was confirmed using the gel shift assay that Cd decreased the transcriptional activity of YY1 in human proximal tubular cells (HK-2 cells). Moreover, mRNA level of UBE2D2 was significantly reduced, but that of UBE2D4 was not changed in YY1-knockdown (KD) cells by siRNA treatment. On the other hand, forkhead box F1 (FOXF1) was detected as the transcription factor whose activity was suppressed by Cd in HK-2 cells using the protein/DNA binding assay. The core sequence for the binding of FOXF1 to DNA (VNDTRTTTRTDYR) was found in the upstream of UBE2D2 and UBE2D4 gene regions. Furthermore, FOXF1-KD decreased UBE2D4 mRNA level but not UBE2D2 mRNA level. Therefore, these results suggest that Cd may down-regulate the gene expression of UBE2D2 and UBE2D4 via suppression of YY1 and FOXF1 transcription factor activities, respectively. [This work was partly supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan]

1533 Cadmium Exposure Influences Angiogenic Pathways in the Preeclamptic Placenta via Epigenetic Mechanisms

S. Brooks. *Environmental Sciences and Engineering, UNC-CH, Durham, NC.*

Preeclampsia (PE), a condition traditionally categorized by high blood pressure and increased protein in the urine of pregnant women, accounts for 40-60% of maternal deaths in the US. PE can also be detrimental to the unborn baby, resulting in premature births, birth deformities, and death. Currently, the etiology of PE is unknown, however, environmental exposures have been linked to placental toxicity/deregulation causing improper vascularization in the placenta. Previously, we have observed a significant correlation between elevated levels of cadmium (Cd) and risk of poor birth outcomes, as well as higher levels of placental Cd and increased odds of developing PE, in women from the southeast US. Furthermore, the transforming growth factor beta (TGF-β) pathway was upregulated in women with preeclampsia compared to those without, further bolstering a connection between vascular deregulation and PE. Investigating the underlying biology of preeclampsia and potential environmental influences can provide insight into the specific effects of toxicants on placental molecular phenotypes that increase risk of PE. The TGF-β pathway is important for regulating several cellular processes in the placenta, such as apoptosis and placental endometrial invasion. Invasion of placental trophoblasts into the uterine wall is substantial for proper placental function and vascularization. We have observed increased gene expression of the TGF-β pathway in preeclamptic women, as well as a decrease in miRNAs that target the pathway, suggesting a potential epigenetic mechanism of control. In addition, trophoblasts exposed to Cd also expressed elevated TGF-β pathway activation, with decreased expression of miRNAs that target the TGF-β receptors and downstream SMADS. Exploring cadmium-induced global expression and epigenetic reprogramming can further enhance our understanding of the genetic interactions that drive preeclampsia.

1534 Effect of Cadmium on p53 Accumulation in Various Cell Lines and Mouse Tissues

M. Satoh, J.-Y. Lee and M. Tokumoto. *Laboratory of Pharmaceutical Health Sciences, Aichi Gakuin University, Nagoya, Japan.*

Chronic exposure to cadmium (Cd) is known to affect adversely to renal functions. Our recent study indicated that Cd induces p53-dependent apoptosis through the inhibition of gene expression of Ube2d family in rat proximal tubule cells (NRK-52E cells)1). In this study, we examined the effects of Cd on p53 protein level accumulation and gene expression of Ube2d family in various cell lines and in kidney and liver of mice. *In vitro*: IEC-6 (rat small intestine epithelial) cells, HBMECs (human brain microvascular endothelial cells) and HBASTs (human brain astrocytes) were treated with Cd. Gene expression was measured by real-time RT-PCR, and protein level was measured by western blotting analysis. *In vivo*: After five-week old female C57BL/6J mice were fed diet containing 300 ppm Cd without restraint for 12 months, kidney and liver were removed from each mouse under ether anesthesia. Gene expression was measured by real-time RT-PCR, and p53 protein level was measured by ELISA. With 5 µm serial sections of kidney and liver, p53 protein was detected by immunohistochemical staining, and apoptosis was detected by TUNEL staining. In IEC-6 cells, Cd decreased not only p53 protein level but also gene expression of Ube2d1, Ube2d2 and Ube2d4. In HBMECs. Cd did not inhibit gene expression of Ube2d family, but increased p53 protein level. In HBASTs, Cd only increased gene expression of Ube2d3. Significant decreases of mRNA levels of Ube2d family and overaccumulation of p53 were detected in kidney of mice exposed to Cd. Decrease of Ube2d mRNA levels in liver was shown in the group of exposure to Cd for 12 months; however, accumulation of p53 in liver was not observed in Cd exposure mice. Interestingly, not only overaccumulation of p53 proteins but also induction of apoptosis was triggered specifically in renal tubules of Cd exposure mice, even in the same spots of serial sections. These results indicate that modification of p53 protein by Cd may have cell variability, and that Cd-induced apoptosis through p53 protein is associated with renal toxicity in preference. [This work was partly supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan]. 1) Tokumoto, et al., (2011) J. Toxicol. Sci., 36, 191-200.

1535 Prophylactic Effects of Coconut (*Cocos nucifera* L.) Water Against Sub-Chronic Low Level Cadmium-Induced Toxicity

O. A. Ogunrinola[1], O. O. Ogunrinola[2], O. S. Shokunbi[1], O. O. Fajana[2] and J. A. Osakwue[2]. [1]*Biochemistry, Babcock University, Ilishan-Remo, Ogun State, Nigeria and* [2]*Biochemistry, Lagos State University OJO, Lagos State, Nigeria.*

Coconut (*Cocos nucifera* L. Arecaceae) Water is an ancient tropical beverage traditionally used for various medicinal purposes and as a natural functional drink. Cadmium (Cd) is a well-known environmentally harmful heavy metal involved in numerous pathological consequences. In the present study, the prophylactic effects of mature coconut water in cadmium-induced toxic rats were evaluated. Sixty-four (64) male Albino rats were randomly divided into eight (8) groups (n=8). Groups 1 and 2 served as controls for both cadmium exposed and coconut water treated groups, while the remaining groups (3, 4, 5, 6, 7, 8) were administered 0.1, 0.2, 0.3 g Cd/L (as cadmium chloride) and coconut water (10%) in their drinking water for 6 weeks respectively. At the end of 6 weeks, all rats were fasted overnight, sacrificed and analyzed for serum alkaline phosphatase (ALP), alanine Aminotransferase (ALT), aspartate aminotransferase (AST) enzyme activities and total bilirubin level spectrophotometrically. Cadmium-exposed rats experienced a steady decrease in weight while the coconut water co-administered animals gained weight significantly ($p<0.05$) throughout the experimental period. Exposure to 0.1, 0.2 and 0.3 g Cd/L cadmium doses resulted in a significant ($p<0.05$) increase in serum ALP, ALT, AST activities and total bilirubin representing 1.4, 3.5, 2.0 and 2.6 folds respectively compared with the control. The co-administration of coconut water with all the cadmium doses resulted in significant ($p<0.05$) decrease of serum ALP, ALT, AST activities and bilirubin. There was a significant up/down-regulation in the AST/ALT ratio from cadmium-induced and co-administrated rats. It can be concluded that, the cadmium had adverse effects on the enzyme activities of ALP, ALT, AST and total bilirubin. Our results indicate that cadmium exposure increases the activities of serum ALP, ALT, AST and total bilirubin level and co-administration of coconut water was able to attenuate related side effects that might develop from this cadmium toxicity.

1536 The Most Scientifically-Supported Approach for Assessing the Carcinogenicity of Oral Exposure to Hexavalent Chromium: Non-Linear, Non-Threshold versus Threshold Approaches

J. T. Haney. *Toxicology Division, Texas Commission on Environmental Quality, Austin, TX.*

Dose-dependent changes in the dose fraction absorbed by target tissues have important implications for the most defensible approach for developing a cancer-based oral toxicity factor for hexavalent chromium (CrVI). The shape of the dose-response curve accounting for the decreasing dose fractions absorbed as doses decrease to lower, more environmentally-relevant doses would be non-linear (i.e., sub-linear). Thus, a non-linear approach, consistent with available mode of action (MOA) data, is most scientifically defensible. Accordingly, building upon previously published studies (Haney, 2015a, 2015b), two non-linear approaches were developed: (1) a non-linear, non-threshold approach; and (2) a non-linear threshold approach. The non-linear, non-threshold dose-response function developed describes the non-linearity predicted (based on previous work) for potential human excess risk versus oral dose due to the sub-linear relationship between oral dose and internal dose across environmentally-relevant doses. For the non-linear threshold approach, benchmark dose modeling was used to derive a reference dose (RfD) with cytotoxicity-induced regenerative hyperplasia as a key precursor event to carcinogenesis in the mouse small intestine. Subsequently, available MOA information was evaluated for selection of the most scientifically-supported approach. The RfD approach is the most scientifically-defensible approach based on the weight-of-evidence (WOE). Furthermore, the RfD derived (0.003 mg/kg-day) shows remarkable agreement with that published previously (0.006 mg/kg-day) based on a more scientifically-sophisticated PBPK approach (Thompson et al., 2013b), as well as the Health Canada (2015) tolerable daily intake for CrVI (0.0044 mg/kg-day) based on the WOE for a threshold MOA. The RfD of 0.003 mg/kg-day may be considered protective of cytotoxicity-induced regenerative hyperplasia as a key precursor event to carcinogenesis and happens to correspond to the approximate human dose at the federal MCL (0.1 mg/L). Despite the WOE for a threshold MOA, if the MOA were actually mutagenic, estimates of the potential risk associated with an RfD of 0.003 mg/kg-day (1.8E-04 to 3E-04) would be near the upper end of acceptable excess risk and lower than the risk associated with the federal MCL for arsenic (5E-04 at the arsenic MCL of 10 ppb based on the USEPA IRIS drinking water unit risk).

1537 Exposure to Cr(VI) Affects Multiple Aspects of Chromatin Dynamics to Attenuate Transcription and Enhance γH2AX Propagation

A. VonHandorf[1], F. J. Sánchez-Martín[2], J. Biesiada[1], M. Medvedovic[1] and A. Puga[1]. [1]*Environmental Health, University of Cincinnati, Cincinnati, OH and* [2]*Molecular Cancer Therapeutics, IMIM Hospital del Mar Medical Research Institute, Barcelona, Spain.*

Two well-established carcinogens with distinct genotoxic mechanisms frequently found in industrial environments are hexavalent chromium and benzo[a]pyrene, an Ah receptor ligand. We have shown in previous research that Cr(VI) treatment limits chromatin accessibility and represses AHR-inducible transcription of Cyp1a1 in mouse Hepa-1c1c7 cells by crosslinking repressive complexes to the promoter. RT-qPCR was used to determine the role of ligands in the transcription-associated responses to Cr(VI) in combination with different AHR ligands. Combinatorial treatment repressed Cyp1a1 and Ahrr, but not Tiparp transcription in a dose dependent manner, suggesting a non-specific Cr(VI)-dependent mechanism affecting poised, AHR-inducible promoters over highly expressed genes. Chronic low-dose Cr(VI) treatment increased levels of the DNA damage signal γH2AX that were not adequately resolved with continued passaging in the absence of Cr(VI), suggesting that chromium exposure has a range of toxic effects capable of disrupting normal chromatin maintenance and accessibility. To determine the extent of H2AX phosphorylation and its connection with initiation of transcription we used ChIP analysis in the genomic region surrounding Cyp1a1 by exposing Hepa-1c1c7 cells to 50 μM Cr(VI) and/or 5 μM B[a]P for 1.5, 4, 8, and 24 hours. Cr(VI) did not induce an increase in γH2AX above control, but enhanced the effect of B[a]P treatment. The AHRE-rich Cyp1a1 enhancer domain showed reduced γH2AX levels and depletion of canonical H2A in the promoter, suggesting this is a region subject to chromatin remodeling events. To investigate Cr(VI)'s toxicity at the level of the nucleosome, Hepa-1c1c7 were exposed to 2 μM K2CrO4 for 72 hours and processed using (MNase)-seq. Positioning and occupancy nucleosome profiles in this region showed that differentially expressed genes exhibited contrasting occupancy profiles at the minus-1 nucleosome, with down-regulated genes showing increased occupancy. Given the recent advances surrounding chromatin architecture within the nucleus, our current research aims to provide a foundation for studying Cr(VI)'s role in architectural aberrations and the possible link to transcriptional dysregulation. Supported by NIEHS R01ES010807.

1538 Chromium in Texas Groundwater—Is Allergic Contact Dermatitis a Risk?

S. E. Ethridge and A. F. Jenkins. *TCEQ, Austin, TX.*

Chromium can elicit both skin irritation and allergic contact dermatitis (ACD). Multiple remediation sites in Texas have identified chromium as a chemical of concern in groundwater, impacting both residential and commercial facilities at concentrations well above the federal Maximum Contaminant Level of 0.1 parts per million (ppm). At many of these remediation sites, dermal contact has been determined to be the major pathway of concern for current use because another source of water is provided or obtained for ingestion. At some of these sites, contaminated water has been used for bathing, hand washing, irrigation, and/or powerwashing. In these cases, the TCEQ Toxicology Division has been asked to determine at what concentration CrVI poses a health hazard via dermal contact. A thorough search of literature and guidance documents did not find a previous quantitative evaluation of the potential risk of ACD from chromium in water. The purpose of this project was to determine a CrVI water concentration that is protective of ACD. We first conducted a thorough literature search for information on the induction and elicitation thresholds for chromium-induced ACD. We then used quantitative risk assessment methodology adapted to address ACD to determine CrVI water concentrations that would be protective of both the elicitation and induction of ACD for specific exposure scenarios. A No Observed Effect Level (NOEL) was identified for induction of ACD, uncertainty factors were applied to the NOEL, and acceptable dermal exposure levels were calculated for each exposure scenario. For elicitation of ACD, a NOEL was not identified due to the large variability in elicitation thresholds for previously sensitized humans. Uncertainty factors were applied to the effect level identified for elicitation of ACD; however, a great deal of uncertainty is associated with the resulting acceptable dermal exposure level. Results of this study indicate that water concentrations of CrVI ≤ 5 ppm are protective of induction of ACD. Much lower CrVI water concentrations could elicit ACD in previously sensitized individuals.

1539 Insights into Chromate Genotoxicity From a One Environmental Health Perspective: Alligators, Turtles, and Whales Oh My!

J. L. Young[2], S. S. Wise[2], C. F. Wise[2], T. Fukuda[1] and J. P. Wise[2]. [1]*Graduate School of Agricultural Sciences, Tohoku University, Sendai, Japan and* [2]*Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Hexavalent Cr(VI) is a human carcinogen and a global marine pollutant. The health impacts of Cr(VI) in marine animals is uncertain. We compared the cytotoxic and genotoxic effects of Cr(VI) in human cells with hawksbill sea turtle (Eretmochelys imbricata), American alligator (Alligator mississippiensis), and fin whale (Balaenoptera physalus) cells. Cr(VI) was cytotoxic and clastogenic to all 4 species. Human cells were most sensitive to the cytotoxic effects of Cr(VI) and whale cells were most resistant. For example, 2.5 uM sodium chromate induced 72, 60, 24, and 4% relative survival, and 1 ug/cm2 lead chromate induced 68, 54, and 10% relative survival in whale, alligator, turtle, and human cells, respectively. Human cells were also the most sensitive and whale cells the most resistant to Cr(VI)-induced genotoxicity. For example, 2.5 uM sodium chromate induced 11 aberrations in 100 whale cell metaphases compared to 26, 35, and 64 aberrations in 100 metaphases in turtle, alligator, and human cells, respectively. Differences in intracellular Cr levels explained the variations in cytotoxicity between cell lines. However, when these differences were adjusted for, alligator cells were the most sensitive to the genotoxic effects Cr(VI). Altogether the data suggest Cr(VI) is a concern for marine animals and that aquatic animals may be good sentinels for the genotoxic impacts of Cr contaminated ecosystems. The data also suggests whale cells may have more efficient cellular mechanisms to protect against chromium induced genotoxicity. This work was supported by the Curtis and Edith Munson Foundation (J.P.W.) and NIEHS grant ES016893 (J.P.W.).

1540 Sodium Dichromate Causes Direct and Persistent Injury to Liver and Kidneys After a Single Intraperitoneal Injection in Male Sprague-Dawley Rats

E. E. Carroll[2] and M. S. Madejczyk[1]. [1]*ORISE Postdoctoral Fellow at US Army CEHR, Frederick, MD and* [2]*Toxicology Portfolio, US Army Public Health Center (Provisional), Edgewood, MD.*

U.S. Service Members and civilians are exposed to a variety of environmental health hazards, including metals used in industrial processes through environmental and occupational exposures. Hexavalent chromium [Cr (VI)], an oxidizing agent and known carcinogen, is extensively used for stainless steel production, chrome plating, and as an anti-corrosive agent. Cr (VI) primarily affects the respiratory system, but can also affect the gastrointestinal tract, liver, kidneys and immune system. In order to protect soldier health from this hazard, additional information on the mechanism of toxicity and biomarkers of exposure and effect are needed. Groups of 7-8 week old male Sprague-Dawley rats were given a single intraperitoneal injection of saline vehicle or increasing doses of Na2Cr2O7, euthanized 1, 3, 7 or 14 days later, and tissues harvested. Histologic examination of liver and kidney revealed direct injury to the capsule and immediately subjacent parenchyma of liver and kidneys, with a slightly delayed response in kidney, possibly due to its retroperitoneal location. Lesions were clearly present 24 hours after injection and persisted as a proliferative capsular fibroplasia with an infiltrate of mononuclear and granular leukocytes, including, in some cases, eosinophils. It is known that prolonged exposure results in pathology, but this may be the first report in rats that suggests persistent injury after a single exposure; underscoring the need for a detection capability to prevent soldier exposure. This research was supported in part by an appointment to the Postgraduate Research Participation Program at the USACEHR administered by the ORISE through an interagency agreement between the US DOE and USAMRMC. Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the U.S. Army.

1541 Toxicogenomic Evaluation of *In Vivo* and *In Vitro* Rat Liver and Kidney Models After Exposure to Cobalt

M. S. Madejczyk[2], M. G. Permenter[1], D. M. Kumsher[1], C. E. Baer[1], W. E. Dennis[3], J. D. Stallings[3] and J. A. Lewis[3]. [1]*Excet, Inc., Fort Detrick, MD;* [2]*ORISE Postdoctoral Fellow at US Army CEHR, Fort Detrick, MD and* [3]*US Army CEHR, Fort Detrick, MD.*

The essential metal cobalt (Co) is widely used in medical devices, dietary supplements and industrial processes providing opportunities for exposure in civilian and military populations. In order to better understand the mechanisms of acute Co toxicity and identify potential biomarkers of exposure or effect, we exposed rats and rat-derived cell lines to cobalt. Rats were orally exposed to three concentrations of CoCl2-6H2O for 1 or 5 days, while cell lines were exposed for 1 day only. Co accumulated in the kidney at both time points in a dose-dependent manner, while there was a significantly larger accumulation in the liver (7.6 µg/kg kidney vs 31.2 µg/kg liver at the highest dose). Co exposure reduced body weight change at both time points in a dose-dependent manner. In addition, we observed a slightly increased incidence of hepatocyte karyomegaly and glycogen depletion, as well as increased serum K+ and Cl- and decreased serum bicarbonate, cholesterol and glucose, suggestive of kidney and liver injury. Changes in liver and kidney gene expression from whole tissues and cell lines were examined using microarrays. Both *in vitro* and *in vivo* differentially expressed genes were enriched with genes involved in oxidative stress and damaged protein responses. Changes in genes for secreted proteins (e.g., Ces and Fbxo30) were identified in both conditions, which could be potential biomarkers. This study provides further insight into the mechanism of Co toxicity and provides a basis for biomarker identification. This research was supported in part by an appointment to the Postgraduate Research Participation Program at the USACEHR administered by the ORISE through an interagency agreement between the US DOE and USAMRMC. Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.

1542 Inhalation Cancer Risk Assessment of Cobalt Metal

G. Brorby[3], M. Suh[2], C. Thompson[1], L. Mittal[1] and D. Proctor[2]. [1]*ToxStrategies, Inc., Katy, TX;* [2]*ToxStrategies, Inc., Mission Viejo, CA and* [3]*ToxStrategies, Inc., Richmond, CA.*

Cobalt, in the form of cobalt metal, salts, hard-metals and alloys, is widely used in a variety of industrial, medical and military applications. Chronic inhalation exposure to cobalt metal and cobalt sulfate have been shown to cause lung cancer in rats and mice, as well as systemic tumors in rats. Epidemiological studies have not found an association between exposure to cobalt metal or salts and increased cancer rates; however, there is evidence of carcinogenicity among workers exposed to hard metals where cobalt, tungsten and carbon are combined. The U.S. Environmental Protection Agency has derived a provisional inhalation unit risk (IUR) value of 0.009 µg/m3 for cobalt based on a chronic inhalation study of cobalt sulfate heptahydrate (a soluble salt) in rodents; however, a recent 2-year study of cobalt metal particulates affords the opportunity to derive IUR values specifically for cobalt metal. Based on benchmark dose modeling of lung tumors in the rodent bioassays, IUR values for cobalt metal ranged from 0.003 to 0.006 µg/m3, indicating that cobalt metal is a less potent lung carcinogen in rats than cobalt sulfate heptahydrate. Multistage and smoothing spline regression modeling gave comparable results, although the latter model predicted more nonlinearity in the low-dose region, which is not evident in the multistage model. *In vivo* and *in vitro* data, including mutation data from the recent bioassay, support that the carcinogenic mode of action (MOA) of cobalt metal in the lung is likely to involve oxidative stress, and thus, the exposure-response may be thresholded. Accordingly, low-dose linear extrapolation from the BMCL10 in high-dose animal studies likely over predicts the risk associated with occupational and environmental exposures. More work is needed to measure and model precursor events, which could support development of a reference concentration (RfC) protective of lung cancer.

1543 Estimating Lead (Pb) Bioavailability in a Mouse Model

D. J. Thomas[2], P. Alava[2], K. Bradham[2], C. Nelson[2] and J. Misenheimer[2]. [1]*US EPA, Research Triangle Park, NC and* [2]*NERL, US EPA, Research Triangle Park, NC.*

Children are exposed to Pb through ingestion of Pb-contaminated soil. Soil Pb bioavailability is estimated using animal models or with chemically defined *in vitro* assays that measure bioaccessibility. However, bioavailability estimates in a large animal model (e.g., swine) can be costly and laborious and bioaccessibility estimates require validation with bioavailability data. Here, we estimated Pb bioavailability from data on tissue Pb distribution after an 8-day exposure of adult female C57BL/6 mice to AIN-93G rodent diet amended with a reference compound, Pb acetate, or NIST-SRM2710a (Montana Soil). At termination, Pb levels were determined in tissues (liver, kidney, blood) in which Pb exerts deleterious effects and bone, the major depot for this metal. For each test material, 4 diets were prepared with Pb concentrations ranging from 3 to 30 µg/g. For Pb acetate or SRM2710a, significant linear relations were found between cumulative amounts of Pb ingested and tissue Pb concentrations. Relative bioavailability (RBA) of Pb in SRM2710a was estimated as ratios of slopes of regression lines describing the relation between cumulative Pb ingestion and tissue Pb concentration for the test materials. This approach yielded 4 estimates of the RBA of Pb in SRM2710a: bone, 0.34; blood, 0.49; kidney, 0.97; liver, 0.65. The estimated mean RBA of Pb was 0.61 (0.27 standard deviation). We also examined relations between Pb concentration in blood and tissues. Here, blood Pb is posited as the proximate source of Pb accumulated by tissues and that the source of Pb in blood (Pb acetate or soil derived) does not affect Pb transfer from blood to tissue. For both test materials, similar linear regression equations described relations between blood Pb concentration and liver or bone Pb concentration. In contrast, the source of Pb in blood affected kidney Pb concentrations. For a given blood Pb level, mice exposed to SRM2710a attained higher kidney Pb concentrations than did mice exposed to Pb acetate. This discordant dosage dependency for tissue Pb distribution should be evaluated in development and validation of the mouse model for Pb bioavailability. (This abstract does not reflect US EPA policy.)

1544 Closing the Ineffective Lead Treatment Gap: Lessons From Hurricane Katrina in New Orleans, USA for Establishing an Effective Lead Intervention Strategy

H. Mielke, C. Gonzales and E. Powell. *Pharmacology, Tulane University School of Medicine, New Orleans, LA.*

Lead is recognized for its many toxic effects on human health and morbidity. Children's clinical blood lead (BPb) is used to diagnose exposure. If BPb is elevated above a specified level (currently 5 mcg/dL) then the prescribed medical intervention requires education and household dust cleanup to reduce exposure. Medical treatments are evaluated by Cochrane Collaboration and review of lead intervention indicates that education and household dust cleanup are not effective at reducing children's lead exposure. Soil lead (SPb) was mentioned but no conclusions were offered. This study relies on changes in New Orleans resulting from the 29 August 2005 Hurricane Katrina (HK) storm-surge and breach of the levee system and flooding of 80% of the city. This event set up a natural experiment whereby SPb and BPb results from before and after the flooding of New Orleans could be compared. The study involved systematically collecting and analyzing soil samples using the same protocols and stratifying the result by census tracts (CT). Before HK New Orleans was surveyed for SPb in 2001; the after HK SPb survey was completed in 2015. BPb results before and after HK BPb was provided by the Louisiana Healthy Homes and Childhood Lead Poisoning Prevention Program. The BPb n's for before HK was 55,551 and 28,868 after HK. Results for before and after HK were matched by CT (n=172). The medians for the entire sample set for before and after SPb results (86 CT in each group) are 283 and 138 mg/kg respectively. For high SPb groups, i.e., equal or above the median, had a SPb median of 552 mg/kg before Katrina and 307 mg/kg after Katrina; BPb associated with the high SPb groups before HK vs. after HK were 6.3 and 2.4 mcg/dL respectively. For the low SPb groups, i.e., below the median, the SPb medians were 86 and 43 mg/kg respectively before and after HK; the median BPb associated with the low SPb groups were 3.6 mcg/dL before HK vs. 1.2 mcg/dL after HK. We conclude that after HK, BPb underwent a remarkable reduction by a factor of at least 2. There was also a reduction in SPb of less than a factor of 2. The before and after HK SPb and BPb data suggests that an alternative intervention for effectively reducing children's BPb is by decreasing the amount of Pb in the soil environment. In the same way that the HK storm surge and construction activities after HK brought in low Pb soil into the city, Pb prevention can be continued by bringing in low Pb soil from outside of the city.

1545 KXRF-Measured Bone Lead (Pb) as a Biomarker for Pb Exposure and Toxicity among Children Diagnosed with Pb Poisoning

A. J. Specht[4], Y. Lin[2], M. Weisskopf[1], C. Yan[2], H. Hu[3], J. Xu[2] and L. H. Nie[4]. [1]*Harvard University, Boston, MA;* [2]*Shanghai Jiao Tong University School of Medicine, Shanghai, China;* [3]*University of Toronto, Toronto, ON, Canada and* [4]*Health and Human Sciences, Purdue University, West Lafayette, IN.*

Childhood lead (Pb) poisoning remains a global issue especially in industrial areas. China, in particular, presents unique exposure issues: pollution, alternative medicines with large amounts of Pb used to treat various diseases, and ever-increasing consumer and industrial usage of Pb containing products. In this study 116 children with an average age of 5.7 years were recruited including 87 patients diagnosed with Pb poisoning and 29 controls from Xinhua Hospital in China. The subjects' bone Pb was measured with a KXRF and a portable XRF system, as well as blood Pb collected for each patient. Pb poisoned patients underwent chelation therapy, and some returned for multiple measurements and treatments. A significant correlation between KXRF bone Pb and blood Pb was observed with an r-squared value of 0.624. A significant correlation between portable XRF and KXRF measurements were also observed, but with a weaker r-squared value of 0.185. The half-life of blood-lead in children was calculated to be 9.96±3.92 days. Our results indicate that: a). bone serves as a storage site for Pb in children and bone Pb is good biomarker to demonstrate total body lead burden; b). bone Pb may be a better biomarker for determination of chelation efficacy especially in children; c). the half-life of blood Pb in children is much shorter than that in adults.

1546 Identification and Characterization of Lactic Acid Bacteria with Lead (II) Biosorption Activity

Y.-J. Yi[2], J.-M. Lim[2], S. Gu[2], W.-K. Lee[1], S.-M. Lee[2] and B.-T. Oh[2]. [1]*College of Veterinary Medicine, Chungbuk National University, Cheongju-si, Korea, Republic of and* [2]*Division of Biotechnology, Chonbuk National University, Iksan-si, Korea, Republic of.* Sponsor: W. Koh.

Heavy metals are essential materials in industry, but their contamination and toxicity can interfere with the biological world. Lead (II) [Pb] had a long history of utilization due to characteristics like malleability, anti-corrosion and low melting point. Although lead is a ubiquitous heavy metal widely used, it is known as a mutagenic and teratogenic substance causing deleterious effects to human and animals. Lactic acid bacteria (LAB) are favorable microorganisms in body, and are known to eliminate metal ions by binding to their cells surface, which is called biosorption. In the present study, lead-resistant LAB were isolated from fermented foods, after then a selected LAB was orally administered to male mice poisoned with lead during the puberty. The detoxification was evaluated in organs and male reproductive function. Total 96 different LAB strains were isolated, and 52 strains showed the lead-resistance. Particularly, L-96 strain indicated remarkable resistance and removal capacity. This strain was identified with Leuconostoc mesenteroides sp. using by PCR-amplified 16s rDNA, and had tolerances to acid and heat. The maximum adsorption capacity of biomass calculated from the Langmuir isotherm was 60.6 mg Pb/g, and lead was mostly attached on the cell surface, which is confirmed by SEM-EDS. In animal experiment *in vivo*, young male mice had water (A), lead-water (B) or lead-water+L-96 (C) during the puberty. There were no significant differences in the weight of body and organs among groups. Higher hepatotoxicity (GOT/GPT) level was shown in group B than those of A and C, and decreased sperm motility and sperm proteasomal-proteolytic activity were observed in epididymal spermatozoa isolated from mice of group B. All female mice were pregnant and gave birth after breeding with males randomly selected from each group. Lower litter size was observed in females mated with males from group B compared to those males of group A and C. In conclusion, isolated L-96 LAB had lead-biosorption activity, and efficiently detoxified male mice poisoned with lead, so that led to normal development of reproductive organ and fertilization competence, suggesting a potential LAB for the environmental protection and human health are needed.

1547 Lipid Alterations in Hepatic and Brain Microsomes Following Subchronic Lead Exposure

E. O. Abam[1,2], O. O. Ogunrinola[1], A. D. Wusu[1], O. K. Afolabi[1], O. A. Dosumu[1], B. O. Onunkwor[1], E. A. Balogun[1], O. O. Odukoya[1] and O. Ademuyiwa[1]. *[1]Biochemistry Department, Federal University of Agriculture, Abeokuta, Nigeria and [2]Chemical Sciences (Biochemistry unit), Bells University of Technology, Ota, Nigeria; [3]Chemistry Department, Federal University of Agriculture, Abeokuta, Nigeria.*

Lead toxicity is still a nagging problem in third-world countries. In order to investigate the effect of sub chronic lead exposure on hepatic and brain microsomes, 24 male albino rats were exposed to 200, 300 and 400ppm lead as lead acetate in their drinking water for 12 weeks. The major lipids, cholesterol, triacylglycerol and phospholipids were determined. Lead was also assayed in the blood, liver, brain, kidney, heart, lungs and spleen. Weight coefficients (organ/body weight ratio) revealed hepatotoxic, cardiotoxic, immunotoxic and pulmonary toxicity potential of lead. 400ppm dose up-regulated liver microsomal cholesterol while both 200 and 400ppm doses down-regulated microsomal triacylglycerol. Liver microsomal phospholipid decreased across all doses (47, 54 and 5% in the 200, 300 and 400ppm doses respectively). Brain microsomal cholesterol increased by 8, 29 and 12% in the 200, 300 and 400ppm doses respectively, while triacylglycerol decreased (8% in 200ppm, 22% in 300ppm and 39% in 400ppm). Brain microsomal phospholipids were down-regulated (25, 21 and 27% in the 200, 300 and 400ppm doses respectively). Alterations in these lipids led to increases in the cholesterol/phospholipid ratios of hepatic and brain microsomes (70, 110 and 20% in 200, 300 and 400ppm doses respectively in the liver and 53, 59 and 82% in 200, 300 and 400ppm doses respectively in the brain). Blood and kidney lead correlated negatively with percentage weight of spleen while blood lead and liver microsomal cholesterol/phospholipid ratio correlated positively. Our findings show that though lead was below detection limit in the spleen, the organ was not spared its toxic effects. Results also confirm the role of microsomal lipids in lead-induced hepatotoxicity and neurotoxicity.

1548 Intellectual Function, Hematological, Hepatic and Molecular Markers in Children Exposed to Lead in Cartagena, Colombia

N. Alvarez-Ortega, K. Caballero-Gallardo and J. Olivero-Verbel. *School of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia.*

Lead produces numerous biochemical and physiological changes in humans, including hematological disorders, effects on the central nervous system and alterations in the function of several organs. The objective of this study was to determine blood lead (BPb) levels in children living in areas near metal foundries occurring in Cartagena, Colombia, associating those with the IQ, hematological and liver damage markers, and the expression of the aminolevulinate dehydratase gene, ALAD. To achieve this purpose, 118 blood samples were collected from children 6–16 years old, with their respective informed consent from their parents. BPb levels were measured by atomic absorption; plasma was utilized to analyze hepatic toxicity markers, ALT, GGT and alkaline phosphatase; the Kaufman Assessment Battery for Children (K-ABC) was administered to measure the IQ; and ALAD gene expression was quantified from blood RNA. The mean blood lead level (BLL) was 1.46 µg/dL. A proportion of the children (3.4%) had BLL above the CDC-recommended limit (5 µg/dL). BPb concentration correlated weakly but negatively with child size, body mass index, hemoglobin, GGT and IQ. ALAD expression was similar between groups classified according BPb readings. These results support the hypothesis that BPb levels below 5 µg/dL may still have a detrimental impact on children's cognitive abilities, development and hematology, supporting recent concerns that there is no safe level of pediatric lead exposure. Colciencias 1107-545-31458 and 567-2012.

1549 Lead-induced DNA Damage: Efficacy of Chelators and Vitamin C

D. O. Adebisi, U. R. Ngozi, O. O. Beno, B. Semiu, O. Temitayo and A. Oladipo. *Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria.*

Lead toxicity resulting from occupational or environmental exposure has long been treated with chelating agents and/or antioxidants. The ameliorative effects of these chelators on the genotoxicity of lead and a possible damage on the DNA are yet to be fully investigated. In this pilot study, we compared the efficacy of conventional chelators (namely; 2, 3-mesodimercaptosuccinic acid (DMSA), D-Penicillamine and CaNa2EDTA with vitamin C in the tissues of experimental rats exposed to lead. A total of 110 albino rats were broadly divided into 3 groups. Control (n=15), administered normal saline for 12 weeks, lead groups (n=55), were administered 150mg/kg body weight lead for 12 weeks and the treatment group (n=40).The control group and a part of lead group (L1) (n=15) were subdivided into 3 groups (12weeks, 12weeks5days and 12 weeks10days). The remaining lead group (L2) was divided into 8 groups (n=5) and were treated with either of Succimer and D-Penicillamine (oral,30mg/kg body weight); vitamin c (oral 150mg/kg body weight; or CaNa2EDTA (IV,30mg/kg body weight) in 2 divided doses of 5 days each. The treatment group was subdivided into 8 groups (n=5) and were treated as control for the L2 group. Twenty four hours after the treatment, animals were sacrificed under anaesthesia and blood was collected by cardiac puncture into heparinsed tube and stored at 40C. The brain, kidney and the liver of the animals were harvested, mopped dry, weighed into aliquots and stored on ice. Quantitative DNA fragmentation was determined spectrophotometically using the diphenylamine reaction to quantify the percentage fragmentation in each of these organs. Results showed a 4-fold increase in the percentage damage observed in the brain of animals administered lead for 12 weeks over that of the control group. Treatment with Succimer and D-Penicillamine for 5 days significantly reduced the fragmentation percentage, while treatment for 10 days was not significantly different from the pattern observed for 5 days. Ten days treatment with vitamin c and the chelators significantly ameliorate the observed injury observed in the DNA of the liver and kidney of the animals, with the most effective reduction found with CaNa2EDTA treated group. The results of the experiment did not suggest oxidative DNA damage resulting from any of the chelating agent nor vitamin c.

1550 Lead and Cortisol Synergistically Accelerate Senescence in Primary Human Umbilical Vein Epithelial Cells

R. Lichtler[2], K. Esteves[2], S. Drury[2], M. Wilson[2] and J. Wickliffe[2]. *[1]Global Environmental Health Sciences, Tulane University, New Orleans, LA and [2]Psychiatry, Tulane University, New Orleans, LA.*

Lead, a well-studied environmental neurotoxin, has recently been associated with wider reaching effects on multiple body systems and molecular structures. In nationwide epidemiological studies, high lead levels have been linked with shorter telomere length (TL). Epidemiological data gathered from children in New Orleans also indicates a correlation between high stress levels and shorter TL in salivary DNA. The purpose of this study was to independently confirm these findings in a controlled, *in vitro* experiment. Primary human Umbilical Vein Endothelial Cells (HUVECs) were exposed to lead acetate (Pb(CH3COO)2) and hydrocortisone (a physiological indicator of stress conditions). qPCR-based TL measurement indicated that when HUVECs are exposed to both lead and hydrocortisone, TL is shorter than when exposed to either compound alone or when left unexposed. After 144 hours in culture, HUVECs exposed to lead and hydrocortisone appeared to have telomeres equivalent to untreated cells cultured for 384 hours. This phenomenon was further confirmed by a senescence marker assay, which showed that cells treated with both lead and hydrocortisone were significantly more senescent than untreated cells. Lead and hydrocortisone appear to work synergistically to promote premature telomere shortening and aging of the cell. On a population level, these results imply that individuals with high social stress levels may have an exacerbated risk for accelerated telomere shortening due to lead exposure.

1551 Intranasal Manganese Exposure: Implications for Adult Neurogenesis and Copper Concentrations in Subventricular Zone

S. L. O'Neal, S. Fu and W. Zheng. *Purdue University, West Lafayette, IN.*

Overexposure to the heavy metal manganese (Mn) occurs in occupational settings, primarily through inhalation. Our previous studies indicate that following subchronic intraperitoneal exposure in a rat model, Mn accumulates in the brain and increases cell proliferation in a region known as the subventricular zone (SVZ), a neurogenic niche that generates neural stem/proliferating cells (NSPC) capable of migrating toward the olfactory bulb. Since inhaled Mn can enter the central nervous system directly via the olfactory pathway, it was interesting to learn if nasal exposure to Mn affects neurogenesis in adult animals. The current study was designed to test this hypothesis with a focus on cell proliferation and differentiation in the SVZ. Anesthetized 8 week old rats were intranasally instilled with saline (as control), 0.2 or 0.8 mg Mn/kg as MnCl2 daily for 14 days. During the last 4 days of Mn exposure, rats also received twice daily intraperitoneal injections of BrdU to label the

NSPC. At necropsy, brains were removed for AAS quantification of Mn and Cu concentrations or prepared for immunohistochemical evaluation of BrdU, DCX, and GFAP in triple-labeled slices to identify newly proliferated cells. In the SVZ, Cu concentrations were significantly reduced (46.4%) following Mn exposure compared with controls ($p<0.01$). Quantification of confocal images revealed significant increases in BrdU (25%, $p<0.05$), DCX (27%, $p<0.01$), and GFAP (27%, $p<0.01$) expression in the SVZ in Mn-exposed animals as compared with controls. Our preliminary data indicate that intranasal Mn exposure perturbs the SVZ's high Cu levels and alters neural cell proliferation critical to the normal adult neurogenesis. The results of this work are significant because they demonstrate a direct interaction of Mn with brain repair mechanisms. (Supported in part by NIH grant ES008146)

1552 Bone Manganese As a Biomarker for Manganese Exposure–An *In Vivo* Pilot Study

Y. Liu, F. Mostafaei, D. Rolle, W. Zheng, E. Wells and L. Nie. *Purdue University, West Lafayette, IN.* Sponsor: W. Zheng.

Occupational exposure to manganese (Mn) occurs among workers involved in welding, smelting, mining, and battery production. In the US alone, millions of workers, including over 300,000 welders, are at high risk of excessive Mn exposure. Workers who have been chronically exposed to excessive amount of Mn can develop severe neurological diseases with Parkinson's like disorders. Until now, there is no reliable biomarker for long term Mn exposure. Mn's long biological half-life as well as its high concentration in bone, makes bone Mn (MnBn) a potentially valuable biomarker for long-term cumulative Mn exposure. In our lab, a neutron activation analysis (NAA) system has been developed and validated for *in vivo*, non-invasive quantification of MnBn in human hand. This system mainly consists of two parts: a compact neutron generator based irradiation system and a high efficiency and resolution high pure germanium detector configuration. Mn-doped hand phantoms were used to calibrate the system. The detection limit was calculated to be about 0.43 µg Mn/g dry bone (ppm) with a radiation dose of 36 mSv to the hand. Approved by Purdue University's IRB, a pilot study was conducted to investigate the association between MnBn and neurological effects. Nineteen volunteers, including some workers who worked with metals, were recruited. *In vivo* NAA system was used to quantify MnBn for these subjects. Purdue Pegboard was used to test the subjects' motor function especially movement coordination. We observed significantly higher MnBn in the subjects who used metal in hobbies or job compared to those who did not (0.53±0.15 ppm vs. 0.25±0.12 ppm). We also observed marginally significant decrease of Purdue Pegboard test scores with the increase of MnBn among the 19 subjects ($p=0.20$-0.40). The results indicate that MnBn may be a good biomarker to assess cumulative Mn exposure and for neurotoxicity study on Mn exposure.

1553 Manganese Levels in Blood and Associated Factors in Urban Population Aged 40 or More in a City in the Southern Brazil

A. L. C. Silva[3], M. R. Urbano[3], M. d. F. H. Carvalho[1], M. L. Buzzo[1], T. Peixe[3] and M. M. Paoliello[3]. [1]*Adolfo Lutz Institute, São Paulo, Brazil;* [2]*Departament of Pathology, Clinical and Toxicological Analysis, State University of Londrina, Londrina, Brazil and* [3]*Graduate Program in Public Health, State University of Londrina, Londrina, Brazil.* Sponsor: M. ASCHNER.

Introduction: Human biomonitoring (HB) studies are used to identify and quantify the exposure and risk to chemical substances. They are useful in order to establish and review occupational exposure limits and to provide data on the exposure in risk assessment processes. Objectives: establish the Mn levels in blood in urban population aged 40 or over in a city in southern Brazil and check for an association between the Mn levels in blood and the social-demographic, lifestyle and high blood pressure variables. Methods: A cross-sectional population-based study was conducted with 958 subjects aged 40 years or older, residing in an urban area in a city in Southern Brazil. Mn concentration in blood was determined by ICP-MS. Systolic and diastolic blood pressure was measured according to the standards set by VI Brazilian Guidelines on Hypertension. Results: The geometric mean and median for Mn in blood in the total population were 12.2 and 12.3 µg/L, respectively. The results showed that the levels of Mn in blood decreased with age. No significant association was found between Mn levels in blood and variables such as gender, smoking habits, alcohol consumption, educational and economic levels, and high blood pressure. Conclusion: This study contributes to the determination of baseline values for Mn in blood in urban population in Brazil, which is pivotal for the identification of population groups at risk, when presenting deviations in relation to the population mean. This study reinforces the preventive character of environmental health.

1554 Tungsten Alters B-Cell Differentiation through Down-Regulation of IL-7R and PreBCR Signaling Pathways

T. H. Wu, A. M. Bolt, D. Plourde, Y. K. Young, A. D. Kelly and K. K. Mann. *McGill University/Lady Davis Institute for Medical Research, Montreal, QC, Canada.*

Tungsten is a metal with desirable properties including strength and flexibility. Increased use of tungsten has resulted in increased human exposure. Recently, high levels of tungsten were identified in the drinking water near three pediatric leukemia clusters, yet the contribution of tungsten to the development of leukemia has not been defined. After an oral exposure of tungsten over 16 weeks, tungsten rapidly accumulated in the bones of mice in a dose-dependent manner. Tungsten exposure correlated with an accumulation of pre-B cells, (fraction CC') and increased DNA damage within the bone marrow. In order to understand the underlying molecular changes caused by tungsten, that result in altered B-cell differentiation; we sorted this pre-B cell fraction by flow cytometry and profiled whole transcriptome gene expression by RNA-Seq from mice with and without a 4-week exposure to 15 ppm tungsten. Interestingly, tungsten down-regulated the expression of multiple genes known to be master regulators of B-cell commitment and development. Tungsten suppressed both the interleukin-7 receptor (IL-7R) and preB cell receptor (preBCR) receptor signaling. These pathways are critical for B-cell differentiation at the pre-B cell differentiation checkpoint, suggesting that tungsten may be stalling B-cell differentiation at this point. Down-regulation of IL-7R and PreBCR signaling were validated in 2 independent RNA samples by Real-Time qPCR, including IL-7R and PreBCR downstream targets: jak1, stat5, ebf1, pax5, syk and ikzf3 (Aiolos). In order to study these signaling pathways and their contribution to tungsten's effects on B-cell differentiation, we are currently developing an *in vitro* primary cell culture model of B-cell differentiation. We will isolate early B cells from murine bone marrow by FACS sorting and differentiate them *in vitro*. At the end of the experiment, B cell differentiation will be monitored by flow cytometry, using a developed multi-parameter flow cytometry panel, based on unique surface makers expressed at each stage of development. Based on our preliminary experiments, tungsten may not be an inert metal as previously described, but may alter B cell lymphopoiesis by downregulating B cell signaling and potentially act as a tumor promoter by stalling B cells in an early stage of development where cells are vulnerable to DNA damage.

1555 Investigating the Effects of Sex and Age of Mice on Tungsten Deposition and Adipogenesis in the Bone

A. M. Bolt, T. H. Wu, D. Plourde, L. F. Negro Silva and K. K. Mann. *McGill University, Lady Davis Institute for Medical Research, Montreal, QC, Canada.*

Tungsten is an emerging environmental toxicant, yet there is limited knowledge of the potential human health risks. Tungsten preferentially accumulates in the bone, where it is a long-term storage reservoir for tungsten, even if the primary source of exposure is removed. There is limited knowledge of the potential effects tungsten has on bone biology. Bone marrow-resident mesenchymal stromal cells (MSCs) can differentiate into multiple cells lineages, including osteoblasts and adipocytes. We have shown that tungsten alters bone biology by promoting adipogenesis in the bone. *In vitro*, tungsten tipped the balance of MSC differentiation by promoting PPARγ-mediated adipogenesis and inhibiting osteogenesis. *In vivo*, tungsten enhanced adipogenesis in the bones of young male mice (5 weeks) exposed to 15 ppm tungsten for 4 weeks. Multiple factors are known to affect adipogenesis in the bone. Both female and older mice have enhanced adipogenesis. In order to evaluate the influence of sex and age on tungsten-induced adipogenesis, we exposed 5-week-old female and 9-month-old male mice to tungsten for 4 weeks. Young female mice had comparable levels of tungsten in the bone to young male mice. Female mice had more adipocytes than their male counterparts, but, tungsten did not further enhance adipogenesis. Conversely, older male mice had no detectable levels of tungsten in the bone. With age, there was an increase in the size of adipocytes, which resulted in a larger area of the bone occupied by adipocytes. However, tungsten did not further enhance adipogenesis in older male mice, which could likely be because there were negligible levels of tungsten in the bone. Interestingly, tungsten increased average adipocyte size in young male mice as compared to the increased adipocyte number observed in female mice. These data suggest that tungsten could be prematurely aging the bone in young male mice, which could have deleterious effects on bone health. Our data indicate that tungsten's effects on bone biology are dependent on both age and sex. Given our data, it would be interesting to investigate the potential roles of sex hormones

and bone formation on tungsten-mediated effects on bone biology, which would provide insight into the underlying molecular mechanisms. In addition, our work helps to identify susceptible subgroups of the population that could be susceptible to tungsten-mediated effects on bone biology.

1556 Alterations in Antioxidant/Oxidant Proteins Following Treatment of Transformed and Normal Colon Cells with Tellurium Compounds

P. Vij and D. Hardej. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Tellurium (Te) is a metalloid, with no known physiologic role in humans. Increasing use of Te compounds in optical blue ray discs and photographic materials suggests that environmental exposure will increase in the future as these products make their way into land fills. Occupational exposure to individuals involved in production of these products may also occur. The neurotoxicity of Te compounds has been documented, particularly in animal studies. However, little has been reported regarding the gastrointestinal toxicity of Te, despite the fact that ingestion represents a likely exposure route. A previous study in our lab has demonstrated that tellurium tetrachloride (TeCl4) causes necrosis and diphenyl ditelluride (DPDT) causes apoptosis via the intrinsic pathway in transformed and normal colon cells. Antioxidant defense systems serve to counterbalance the effects of oxidants and prevent oxidative damage. The purpose of the current study was to evaluate the potential of tellurium compounds TeCl4 and DPDT to induce oxidative stress by examining the protein levels of cyclooxygenase-2 (COX-2), glutathione reductase (GR) and NADPH dehydrogenase quinone 1 (NQO1) in CCD-18Co cells and cytoglobin (CYGB) and neutrophil cytosolic factor-1 (NCF-1) in HT-29 cells. Upregulation of these respective genes were also noted in qPCR in both the cell lines. A significant increase in COX-2 activity was observed at concentrations of 500 µM-1000 µM DPDT and 125 µM-1000 µM TeCl4 after 24 hr exposures. Significant increase in NQO1 was also observed at concentrations of 500 µM-1000 µM of DPDT and TeCl4 in CCD-18Co cells. In HT-29 cells, increase in Cygb was noted at concentrations of 500 µM-1000 µM DPDT and TeCl4 while NCF-1 increases were noted at the 1000 µM concentration of DPDT and TeCl4. In conclusion, proteins levels of COX-2, and NQO1 were upregulated in HT-29 cells and NCF-1 and Cygb were upregulated in CCD-18Co.

1557 Protective Effect of Ethanolic Extract of *Grewia carpinifolia* Leaves on Vanadium-Induced Toxicity

O. E. Adebiyi[2], A. Obisesan[2], F. O. Olayemi[2] and J. O. Olopade[2]. [1]*Veterinary Anatomy, University of Ibadan, Ibadan, Nigeria, Oyo State, Nigeria and* [2]*Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria, Oyo State, Nigeria.*

Pentavalent vanadium (metavanadate salt) toxicity is a challenging environmental hazard that affects living organisms. Studies have shown that plants play important roles in protecting against heavy metal toxicity. This study was designed to evaluate the protective activity of ethanolic extracts of *Grewia carpinifolia* leaves following vanadium toxicity. Twenty five male Swiss mice were randomly divided into five groups (A-E) of five rats each. Group A rats served as control and were given distilled water, Group B was administered with sodium metavanadate and a known antioxidant agent; α-tocopherol, Groups C and D were administered with sodium metavanadate and ethanolic extract of *Grewia carpinifolia* leaves orally at 100 and 200 mg/kg body weight respectively while Group E was administered with only sodium metavanadate. After a daily single oral dosing for seven days, changes in behaviour, haematology and serum biochemistry parameters were analysed. Sodium metavanadate caused a significant decrease ($p \leq 0.05$) in haematocrit levels, haemoglobin (Hb) concentration, white blood cell count, neutrophil count and serum cholesterol level. A significant ($p \leq 0.05$) lymphocytosis was also observed in the group administered with sodium metavanadate alone. *G. carpinifolia* extract given concomitantly with sodium metavanadate was able to restore PCV, Hb concentrations and serum total protein to levels comparable with the control and standard groups. *Grewia carpinifolia* also significantly reduced the elevated serum levels of AST and ALT after vanadium induced hepatotoxicity. Our findings suggest that *G. carpinifolia* extract protected against the toxicity induced by vanadium; the plant extract at 200 mg/kg however appear to offer a better protection.

1558 Comparison of the Toxicity of Sintered vs. Unsintered Indium-Tin Oxide Particles on Murine Macrophage and Epidermal Cells

N. S. Olgun, A. M. Morris, A. B. Stefaniak, K. J. Cummings, M. L. Kashon and S. S. Leonard. *Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, WV; Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV.*

Revenues from consumer electronics are at an all-time high, leading to an increased demand for indium-tin oxide (ITO) to produce touch screens and plasma and liquid crystal displays. Composed of 90% indium oxide (In2O3) and 10% tin oxide (SnO2) by weight, ITO is synthesized under conditions of high heat via a process known as sintering. Concerns have arisen over the health of workers in the ITO industry, as severe pulmonary toxicity and increased levels of indium in blood have been associated with occupational exposure to ITO. In the current study, murine macrophage (RAW 264.7) and epidermal (JB6/AP-1) cells were used to differentiate between the toxicological profiles of sintered ITO (SITO) and an unsintered ITO (UITO) mixture. We hypothesized that sintering would play a key role in free radical generation and cytotoxicity. Cells were treated with either 50 µg/ml, 150 µg/ml or 250 µg/ml of ITO and various endpoints were measured over time. Exposure of cells to both UITO and SITO caused a time and dose dependent decrease on the viability of cells. Intracellular ROS generation was inversely related to the concentration of both UITO and SITO, a direct reflection of the decreased number of viable cells observed at higher concentrations. Electron paramagnetic resonance, used to measure free radical generation, showed significantly increased hydroxyl radical generation in cells treated with UITO versus those treated with SITO. This is different from observed LDH release, which showed that SITO caused significantly increased damage to the cell membrane compared to UITO. Our results delineate the need for a better understanding of the mechanisms of toxicity and free radical production associated with workplace exposure to indium, allowing for earlier disease detection and improved health amongst workers.

1559 Prevalence of Anti-Nuclear Antibodies Among Navajo Birth Cohort Study Participants with Elevated Urine Uranium Levels

D. MacKenzie, E. Erdei, J. Ong, B. Pacheco, C. Miller and J. Lewis. *Pharmaceutical Sciences-Community Environmental Health Program, University of New Mexico College of Pharmacy, Albuquerque, NM.*

Native American Tribal communities in the United States are concentrated in the Western US, a region home to the highest concentration of abandoned hardrock mines (over 161,000) in the country. The Navajo Nation currently has more than 500 abandoned uranium mines. The Navajo Birth Cohort Study (NBCS) is an ongoing prospective study enrolling mothers, fathers, and babies to investigate the impact of environmental exposure to metals found in mine wastes on pregnancy, birth outcomes and early child development. NBCS participant samples were analyzed for 36 metals at the CDC Laboratory in Atlanta GA. To date, 85.9% of tested samples (N=455) have urine uranium above the NHANES 50th percentile (i.e. >0.006µg/L) and 24.4% have levels greater than the 95th percentile (i.e. >0.031 µg/L). Development of anti-nuclear antibodies (ANA) has been associated with environmental exposure to metals, and increased levels of maternal ANA with or without a maternal autoimmune disease diagnosis have been associated with development of autism spectrum disorders in children, providing a potential link between environmental exposures, ANA, and child development. Serum samples from 80 NBCS participants (40 male, 40 female) were tested for ANA using a microparticle immunoassay for 10 common autoantigens (centromere B, ribosomal P, chromatin, ds-DNA, Smith, mRNP, SS-A, SS-B, Scl-70 and Jo-1). The ANA prevalence for the US as a whole is 13.8%, and more frequent in females and older individuals. Within this young cohort (average age of 27), 18.8% of the samples (15/80) were ANA(+), with equal distribution across gender. Statistical analysis showed an interaction between urine uranium, gender and ANA, with a positive relationship for both genders, though stronger in males ($p=0.05$), suggesting a relationship between environmental exposure to uranium and induction of ANA. Development of ANA can precede clinical disease diagnosis by many years, making ANA a potential biomarker of metal exposures and providing important insights into mechanisms of metals-induced immunotoxicity such as immune dysregulation, oxidative stress and/or DNA damage. These findings warrant additional investigation of mixed metal exposures in addition to uranium within this young population.

1560 Environmental Uranium Exposure and Immune Biomarkers Among Younger Generations of Navajo Tribal Members

E. Erdei, D. MacKenzie, C. Miller, B. Pacheco, J. Hoover, J. Ong and J. Lewis. *Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

Since 1986, un-remediated uranium mining and milling operations left a legacy of environmental uranium and metal mixture exposures on the Navajo Nation. Navajo communities and the Tribal leadership are concerned that increased environmental metal exposures, particularly to uranium, significantly influence poor health outcomes. The Navajo Birth Cohort Study examines the impact of uranium exposures on pregnancy, birth outcomes and early child development among Navajo women in child-bearing age and their babies. This ongoing study is assessing exposures through metal analysis of biological specimens, in-home assessments of dust samples and use of geospatial statistics to identify exposure "hot spots." The NBCS has already enrolled 561 mothers and follows 392 babies. Our current biomonitoring data demonstrates significant uranium exposures, with 85.9% of the young Navajo adults and 28% of the newborn babies are above the 50th percentile of NHANES for urine uranium levels. To begin to assess the immunological consequences of these exposures, we measured serum concentrations and determined pattern of cytokine productions using a 13-plex cytokine/chemokine panel (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, INF-γ, TNF-α, and GM-CSF) using magnetic bead multiplexing assay (Milliplex, EMD Millipore) in 53 mother and newborn baby pairs with either low (below the 50th percentile of NHANES) or high (above 50th percentile of NHANES) urine uranium exposures. Based on descriptive statistical information, baby cord blood samples had significantly more values below the detection limit compared to their mother's samples. However, the babies' levels of IL-4, IL-6, IL-8 and TNF-α were several times that of the adult concentrations. Increased levels in newborns are considered normal and might even necessary at birth to prime healthy immune response. Importantly, the detectable IL-13 levels among babies and the pattern of inflammatory response among NBCS mothers warrant detailed mixed modeling approach for identifying these outcomes with increased levels of excreted uranium and *in utero* exposures.

1561 Lung Function in Gulf War I Veterans Exposed to Depleted Uranium Inhalation and Shrapnel Injury: Updating the Cohort

S. E. Hines[1,3], A. Barnes[3], P. Gucer[1,3], J. Gaitens[1,3], M. Oliver[1,3], M. Condon[1,3], T. Roth[1,3], K. Squibb[1,3] and M. McDiarmid[1,3]. [1]*Baltimore VA Medical Center, Baltimore, MD;* [2]*University of Maryland School of Medicine, Baltimore, MD and* [3]*Division of Occupational and Environmental Medicine, University of Maryland School of Medicine, Baltimore, MD.*

Rationale: A cohort of Gulf War I (GWI) Veterans who sustained traumatic injury and inhalational exposure to depleted uranium (DU) following friendly-fire incidents in 1991 are surveyed biennially for long-term health effects related to DU. Veterans who have retained shrapnel have persistently higher urine uranium (uU) levels than other cohort members who had only an inhalation exposure. The shrapnel sub-group may have also experienced greater inhalational doses. Due to potential respiratory effects from inhalational exposure to DU and blast particulate, we continue to evaluate this cohort for the presence of lung function abnormalities. We hypothesized that Veterans with higher DU body burdens would have more abnormalities in spirometry compared to those with lower U burdens. Methods: 36 members of a dynamic cohort of 84 GWI Veterans with known DU exposure were seen in biennial surveillance between April-June 2015. All completed a medical history and exposure questionnaire, 24 hour urine collection for U concentration, and pre-bronchodilator spirometry. We evaluated mean pulmonary function parameters, including Forced Expiratory Volume in 1 second (FEV1), Forced Vital Capacity (FVC), and the ratio of FEV1/FVC. We compared outcomes in Veterans with higher uU versus lower uU content. Results: Participants in the high versus low U groups did not differ by age, race, BMI, or ever-smoking status. Mean spirometry values overall fell within the normal range. For the high versus low U groups, mean % predicted values were: 94.5 vs. 93.5 for FEV1; 89.5 vs. 88.19 for FVC. Mean FEV1/FVC ratios for the two groups were not reduced and were similar: 77.4% for the high U group, and 77.8% for the low U group. Conclusions: The presence of higher systemic U body burden is not associated with worsened spirometric indicators of lung function. Lung function in the cohort overall remains normal, 24 years post initial insult, with no indication of U-specific association. Future work will incorporate Impulse Oscillometry evaluation to determine whether differential effects on the small airways can be seen as a function of U burden. Funded by the US Department of Veterans Affairs.

1562a Comparative Study of *Chlorella vulgaris* and *Phanerochaete chrysosporium* (ATCC® 24725 TM) on the Bioremediation Hexavalent Chromium: A Mathematical Modeling Study

C. Mwase, B. Mohanty and C. P. Abubucker. *Department of Mathematics, Science, and Technology, Paine College, Augusta, GA.* Sponsor: T. Burns.

Chromium (Cr) is a transition metal that is widely used in industrial processes such as in the manufacturing of pigments, dyes, and electroplating. Compounds containing the +6 state are more toxic due to their solubility in water and permeability through membranes. Hexavalent chromium (Cr (VI)) is mutagenic as well as carcinogenic to humans, animals, and plants. Individuals who work in industrial chrome plants or live near disposal areas are at risk of exposure to Cr (VI). Currently, methods used to remove heavy metals such as chromium include ion exchange, precipitation, and reduction-oxidation. These methods are very expensive and tend to generate more hazardous wastes. The binding of metals to microbial cells, or biosorption, can be used as an alternative low cost treatment option for heavy metal removal from the environment. Treatment by biosorption is less expensive and would minimize the generation of hazardous wastes. Previously we examined Cr (VI) removal by *Chlorella vulgaris* (green algae). In this study, Cr (VI) biosorption by *Phanerochaete chrysosporium* (fungi) was investigated. We hypothesized that the mycelilal biomass of *P. chrysosporium* would possess greater adsorptive capacity of Cr (VI) than the biomass of *C. vulgaris* (green algae). To investigate this, *P. chrysosporium mycelia* grown in liquid Potato Dextrose Agar (PDA) for 10 days were washed and homogenized before being treated with different concentrations of Cr (VI) (2, 4, 6, 8, 10, 20, 30, and 40 mg/L). These were incubated for different time intervals (1, 2, and 4 hours). The Cr (VI) was analyzed spectrophotometically at 540 nm using the 1,5-diphenylcarbazide method. The mechanism of adsorption was evaluated by analyzing the data using the Langmuir and Freundlich mathematical models. We found that Cr (VI) adsorption by *P. chrysosporium* was greater than that of *C. vulgaris*. The study suggests that *P. chrysosporium* may have a greater potential in developing low cost cleanup technology, especially for low-level contaminations of hexavalent chromium.

1562 SATB2 Plays a Role in Anchorage -Independent Growth and Cell Migration in Normal and Metal Transformed Human Bronchial Epithelial Cells

A. Jordan[2], F. Wu[2], T. Kluz[2], S. Shen[1], H. Sun[2], L. A. Cartularo[2] and M. Costa[2]. [1]*Center for Health Informatics and Bioinformatics, New York University, New York, NY and* [2]*Environmental Medicine, New York University, Tuxedo, NY.*

Given the significant gaps in our knowledge of metal induced carcinogenesis, there is a great need to elucidate the mechanisms but which this process occurs. Our lab has previously shown that chronic treatment of bronchial epithelial cells (Beas2B) with several known carcinogenic metals can transform normal cells and simultaneously increase the expression of the special AT-rich sequence binding protein 2 (SATB2). This finding indicated that SATB2 is possibly an important factor underlying metal induced carcinogenic transformation. The SATB2 gene encodes a protein transcription factor that regulates gene expression by changing DNA chromatin structure and has been implicated in a number of cancers. In this study, we showed that over expression of SATB2 in Beas2B cells increased anchorage independent growth while conversely, shRNA mediated SATB2 knockdown decreased anchorage independent growth in nickel transformed Beas2B cells. In addition, RNA sequencing of SATB2 over-expressing Beas2B cells revealed enrichment in genes involved in cell-movement and adhesion pathways. Taken together, our findings suggest that SATB2 plays an integral role in Beas2B carcinogenic transformation. Acknowledgements: This study was supported by National Institutes of Health grants RO1 ES022935, ES023174 and National Institute of Environmental Health Sciences grant P30ES000260.

1563 Activation of Nrf2 Through S-Transmerculation of Methylmercury Glutathione Adduct to Keap1 in SH-SY5Y Cells

Y. Abiko[2], E. Yoshida[2] and Y. Kumagai[2]. [1]*Faculty of Medicine, University of Tsukuba, Tsukuba, Japan and* [2]*Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan.* Sponsor: Y. Kumagai.

Methylmercury (MeHg) reacts readily with glutathione (GSH), leading to the formation of a methylmercury glutathione adduct (MeHg-SG) that is excreted into extracellular space through a multidrug-resistance-associated protein (MRP), which is regulated by the transcription factor Nrf2. We previously reported that MeHg covalently modifies Keap1 protein and activates Nrf2 in human neuroblastoma SH-SY5Y cells. In the study presented here, we examined whether or not the MeHg-SG adduct could also modulate the Keap1/Nrf2 pathway, because the formation of the Hg-S bond is believed to be reversible in the presence of a nucleophile. SH-SY5Y cells exposed to the synthetic ethyl monoester of the MeHg-SG adduct (which is hydrolyzed by cellular esterase(s) to give the MeHg-SG adduct) exhibited a concentration-dependent cellular toxicity that was enhanced by pretreatment with a specific MRP inhibitor. As expected, the MeHg-SG adduct was able to modify cellular proteins in the SH-SY5Y cells and purified Keap1. We also found that this pro-drug, as well as MeHg, causes the cellular Keap1 in the cells to be modified, resulting in Nrf2 activation and, thereby, the up-regulation of the downstream genes. These results suggest that the MeHg-SG adduct is not electrophilic, but modifies protein thiols (including Keap1) through S-transmercuration, and that rapid Nrf2-dependent excretion of the MeHg-SG adduct is essential in decreasing the cytotoxicity of MeHg. Yoshida, E., Abiko, Y., Kumagai, Y. Chem. Res. Toxicol. 27, 1780-6 (2014) Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (#25220103 to YK)

1564 Role of MRTF, a Novel Transcription Factor, in Induction of TNF-α Gene Expression by Methylmercury

N. Endo, A. Naganuma and G.-W. Hwang. *Tohoku University, Sendai, Japan.* Sponsor: A. Naganuma.

Methylmercury is an environmental pollutant known as a causative agent of Minamata disease. However, the molecular mechanism about the methylmercury toxicity is still unclear. We recently found that MRTF (Methylmercury Responsive Transcription Factor) might be involved in the enhancement of methylmercury toxicity in HEK293 cells through the increase of TNF-α gene expression and its extracellular release. Thus, in this study, we examined the role of MRTF in methylmercury toxicity enhanced by the increase of TNF-α gene expression. We first examined the levels of MRTF mRNA and protein, and found that the both levels were increased in HEK293 cells treated with methylmercury. Since the level of MRTF protein was not increased by methylmercury in presence of a mRNA synthesis inhibitor, acceleration of transcription of MRTF might be mainly involved in the increase of its protein level by methylmercury. In previous study, we found that the increase of TNF-α gene expression by methylmercury was abrogated with knockdown of MRTF. Therefore, we examined the effect of methylmercury on recruitment of MRTF to the TNF-α promoter region using DNA-protein binding assay, and found that the binding level of MRTF to the TNF-α promoter region was significantly increased by treatment with methylmercury. MRTF has a homeodomain which is known to promote transcription by connected in specific motif. However, this motif was found to not be involved in the induction of TNF-α by methylmercury via MRTF by domain analysis. We next established MRTF-overexpressing cells, and found that an overexpression of MRTF induced TNF-α gene expression and inhibited the cell proliferation. This inhibition was partially improved by treatment with a TNF-α antagonist, suggesting that TNF-α is involved in part to the inhibition of cell proliferation by MRTF overexpression. Our findings suggest that MRTF is induced by methylmercury and might contribute to the development of methylmercury toxicity as a novel transcription factor involved in TNF-α gene expression.

1565 Developmental Exposure to MeHg Inhibits Myogenic Differentiation in C2C12 Myoblasts

L. Prince and M. D. Rand. *Environmental Medicine, University of Rochester, Rochester, NY.*

MeHg is a ubiquitous environmental toxicant, and fish consumption is the main route of exposure. Uncertainty in the relative risk of MeHg exposure versus the benefits of fish nutrients remain, specifically with respect to identifying sensitive populations. Gaining a full understanding of whom is more susceptible to MeHg and why is imperative. MeHg exposure can cause both motor and cognitive defects, with children exposed *in utero* being most vulnerable. Decades of studies have focused on MeHg as a developmental neurotoxicant, but few have examined the neuromuscular system in its entirety. Data from our lab has shown that MeHg disrupts development of both neurons and muscles in Drosophila and preferentially upregulates a Notch target gene that is predominantly expressed in developing muscles. We further demonstrated that MeHg tolerance and susceptibility in developing flies associates with core genes in pathways of muscle and neuromuscular development, including components of Notch signaling. Notch signaling is a conserved pathway for directing myogenesis. Therefore, we hypothesized that MeHg can impact developing muscle by upregulating Notch target genes involved in myogenesis. We tested this hypothesis by exposing differentiating C2C12 mouse myoblasts to MeHg and analyzing muscle cell morphology. We also investigated dose-dependent effects of MeHg on gene expression levels of the Notch receptor, its downstream targets, and myogenic genes Pax7 and myogenin. Chronic and acute MeHg exposures reduce myotube formation and decrease the ability of the myoblasts to differentiate, as seen by an increase in Pax7, a marker of undifferentiated myoblasts, and a decrease in myogenin, a marker of differentiation. This reduction in myoblast differentiation may be due to a MeHg-induced modulation of Notch target genes. Future studies aim to investigate the role of Notch signaling as a mediator of MeHg myotoxicity, both in a mammalian model and in Drosophila. We anticipate our findings will advance the understanding of the role that muscle tissue plays as a developmental target for MeHg toxicity. Funded by NIEHS RO1 ES07026 and NIEHS P30 ES001247.

1566 Methylmercury Induces Cytotoxicity Through Transport of Pyruvate into Mitochondria

J.-Y. Lee[1,2], Y. Ishida[1], A. Naganuma[1] and G.-W. Hwang[1]. [1]*Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan and* [2]*School of Pharmacy, Aichi Gakuin University, Nagoya, Japan.*

Methylmercury (MeHg) is a major environmental pollutant. However, little is known about the molecular mechanisms of MeHg toxicity and cellular protective mechanisms against such toxicity. We have previously reported that overexpression of enzymes involved in the production of pyruvate, enolase 2 (Eno2) and D-lactate dehydrogenase (Dld3), rendered yeast highly sensitive to MeHg, and that promotion of intracellular pyruvate synthesis may be involved in enhancement of MeHg toxicity. In this study, we showed that pyruvate added to culture media in non-toxic concentrations enhanced the sensitivity of yeast and human neuroblastoma cells to MeHg. We also found that MeHg promoted the transport of pyruvate into mitochondria, and increased pyruvate concentrations in mitochondria were thus involved in enhancement of MeHg toxicity without pyruvate being converted to acetyl-CoA. Meanwhile, in human neuroblastoma cells, MeHg treatment alone decreased the mitochondrial membrane potential, and pyruvate addition led to a further significant decrease. In addition, N-acetylcysteine (antioxidant) treatment significantly reduced the MeHg toxicity and significantly inhibited the intensification of MeHg toxicity by pyruvate. Based on these data, we hypothesize that MeHg exerts its toxicity by increasing the level of pyruvate in mitochondria, and that mitochondrial dysfunction and increased levels of reactive oxygen species are involved in the action of pyruvate. Our present findings suggest the existence of a novel MeHg toxicity mechanism targeting mitochondria. The mechanisms related to MeHg toxicity may be clarified by studying in detail the relationship between the level of pyruvate in mitochondria and the known toxic mechanism of MeHg through mitochondria.

1567 Total Mercury and Methylmercury Analysis in the Muscle and Gonads of Seafoods Using Heating Vaporization Atomic Absorption Spectrometry

K. Yoshimoto[1,3], A. Yamamoto[5], C. Koriyama[5], Y. Ishibashi[1], M. Tabata[2], A. Nakano[3] and M. Yamamoto[3]. *[1]Prefectural University of Kumamoto, Kumamoto, Japan; [2]Saga University, Saga, Japan; [3]Department of Basic Medical Science, National Institute for Minamata Disease, Minamata, Japan; [4]Graduate School of Fisheries, Kagoshima University, Kagoshima, Japan and [5]Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan.*

To develop an easy and cost-effective analytical method for total mercury (T-Hg) and methylmercury (MeHg) in biological samples with reproducibly high recovery of MeHg using heating vaporization atomic absorption spectrometry, we examined the pretreatment process of biological samples such as solubilization, degreasing and solvent extraction of MeHg using the Magos method. We regarded organic mercury determined in this method as MeHg, because MeHg is the predominant form of organic mercury in most human samples and seafoods. Samples were solubilized with NaOH at 80°C and degreased of fat components by methyl isobutyl ketone and hexane. HBr was used to acidify the solubilized solution to form MeHgBr with high solvent transferability. The fraction containing MeHg-L-cysteine complexes in aqueous solution, which had been reverse-extracted from the toluene layer, were determined as MeHg. Recoveries of MeHgBr were ≈95 % with little variation. Analytical values for standard reference materials for hair and fish using the present method agreed well with certified values. Next, this method was applied to determine T-Hg and MeHg concentrations in the muscle and gonads of seafoods. The MeHg/T-Hg ratios of muscle in red seabream and golden threadfin bream were in the range of 96-98%. The MeHg/T-Hg ratios in the testis and ovary of red seabream were 46.6% (from 7 of 10 samples) and 42.4% (3/10), respectively. The MeHg/T-Hg ratio in the testis of golden threadfin bream (n=10) was 80%. These results indicate that this method can measure both T-Hg and MeHg using the same biological sample in one consecutive step by a single apparatus, and is applicable to conduct the risk assessment of MeHg exposure derived from seafoods.

1568 Effect of Low Level Sub Chronic Inorganic Mercury Exposure on the Level of Essential Elements Analysed by ICP-MS in Rat Blood

A. D. Wusu[3], O. O. Ogunrinola[3], O. K. Afolabi[2], E. O. Abam[4], D. O. Babayemi[1], O. A. Dosumu[1], O. B. Onunkwor[1], E. A. Balogun[5], O. O. Odukoya[1], T. C. Onwordi[3] and O. Ademuyiwa[1]. *[1]Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria; [2]Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Nigeria; [3]Biochemistry, Lagos State University, Lagos, Nigeria; [4]Biochemistry, The Bell University, Ogun, Nigeria and [5]Biochemistry, University of Ilorin, Ilorin, Nigeria; [6]Chemistry, Federal University of Agriculture, Abeokuta, Nigeria; [7]Chemistry, Lagos State University, Lagos, Nigeria.*

The interaction of toxic metals with essential elements could lead to disruption in the homeostasis of essential metals. The study investigated the effect of low level exposure of inorganic mercury to essential elements (Zn, Fe, Se, Mg and Ca) of male and female rats. The rats were exposed to 0.5, 1.0 and 1.5 mg/kg of Hg concentrations for 12 weeks after which blood, liver, kidney, brain, spleen, heart and lungs were removed from the animals and these elements were determined by inductively coupled plasma-mass spectrometer (ICP-MS). The result showed that mercury accumulated in all the organs but more mercury uptakes were observed in the kidney than every other tissues, markedly in females (77.89±7.7 and 23.78±1.59 µgHg/g). The exposure resulted in decrease in Mg, Ca, Fe, and Zn in nearly all the tissues except for brain and liver where Fe increase significantly in both sexes compare to controls($p<0.05$). Meanwhile, Se behave differently in kidney and blood, in terms of its mutual competition with Hg. The increase in Hg dose resulted in gradual and significant increase in the accumulation of Se in kidney and blood with gender significant. There was also significant increase in Ca and Mg in lungs of both sexes. No variation was observed with zinc in brain and liver. Most notably, Hg correlated negatively with Ca and Mg. These findings indicate the importance of the balance between pro-oxidants and antioxidants

1569 Associations of the Gut Microbiome with Methylmercury Biotransformation and Elimination in Humans Following Fish Consumption

S. W. Caito[3], D. Vorojeikina[3], A. Grier[3], S. Gill[3], E. van Wijngaarden[3] and M. D. Rand[3]. *[1]Environmental Medicine, University of Rochester, Rochester, NY; [2]Microbiology and Immunology, University of Rochester, Rochester, NY and [3]Public Health Sciences, University of Rochester, Rochester, NY.*

Methylmercury (MeHg) is a known neurotoxin present in fish and seafood, whose elimination from the human body remains poorly understood. Attempts to characterize MeHg elimination have revealed the wide variation in whole body half-time (t1/2) that exists among individuals and populations ranging from 35 to >120 days. It has previously been observed that antibiotic treatment significantly alters the elimination of MeHg in rodents. Additionally, some strains of bacteria are known to harbor Hg resistance genes that encode enzymes capable of mediating the biotransformation of MeHg into the more readily excretable inorganic Hg. We hypothesized that differences among individuals' gut microbiomes may account for differences in whole body elimination rate and de-methylation of MeHg. Elimination rates in eight individuals were determined following the consumption of three fish meals by longitudinal Hg analysis in hair using a laser ablation-inductively coupled plasma-mass spectrometry method. De-methylation of MeHg was estimated from levels of total and inorganic Hg measured by cold vapor atomic absorption in fecal samples collected following the fish meals. The data demonstrated a wide range in MeHg elimination rates (t1/2=42.5 to 128.3 days). We also found that the ratio of MeHg and inorganic Hg in feces varied among individuals, indicating different degrees of MeHg de-methylation. Analysis of the gut microbiome of each subject at time points prior to and subsequent to fish consumption was performed by Illumina MiSeq sequencing of total 16S ribosomal DNA isolated from fecal samples. Fish meal consumption did not associate with an overall change in diversity within each individuals' microbiome. Analysis of operational taxonomic units revealed that the abundance of multiple taxa are significantly associated with MeHg clearance rates, the most significant being Firmicutes of the order Clostridiales. Finally, using quantitative PCR, we identified the presence of two bacterial genes in the fecal DNA samples that are predicted to be involved in de-methylation of MeHg. Collectively these data implicate a role for variation in the gut microbiome composition in modulating the biotransformation and elimination of MeHg in humans.

1570 Wheat Bran Enhances Urinary Elimination and Reduces Mercury Levels in Blood and Brain After Methylmercury Exposure in Mice

M. Nagano[2], M. Fujimura[2] and K. Inaba[1]. *[1]Azabu University, Sagamihara, Kanagawa, Japan and [2]National Institute for Minamata Disease, Minamata, Kumamoto, Japan.* Sponsor: M. Yamamoto.

Methylmercury (MeHg) is a neurotoxicant that exist widely in the natural environment. Since MeHg is accumulated in seafood through the food web, consumption of fish and shellfish is major source of MeHg exposure for humans. Because of fears regarding the adverse effect of MeHg contained in seafood on the developing fetus brain, pregnant women have been warned against consuming fish and shellfish in many places such as the United States and Europe. In recent years, it has been reported that fruit consumption may provide a protective effect for Hg exposure in Amazonian riparians. Although it has revealed that the feeding of diet containing 30% wheat bran decreased the total Hg concentration in the brain and blood of MeHg-treated mice, it is unclear for its mechanism. To reduce toxic risk by the function of food, we investigated the effect of wheat bran on Hg accumulation after a single administration of MeHgCl (5 mg MeHgCl/kg). Female BALB/c mice were housed in the metabolic cages and fed containing 0% or 30% wheat bran in a basal diet (AIN-76). Then urine and feces were collected for 4 weeks. At the end of the experiment, mice fed the wheat bran diet showed lower total Hg levels in blood, brain, kidney than animals fed the basal diet. 4 weeks after MeHg administration, the cumulative Hg excretion in urine was increased markedly by wheat bran diet-fed mice, whereas fecal Hg excretion increased slightly. These results suggest that wheat bran accelerates urinary Hg excretion and subsequently decreases tissue Hg levels in mice. LC-MS analysis showed the oxidized forms of glutathione and Γ-glutamylcysteine characteristically contained in the wheat bran diet. Also, the analysis of diet compositions were found that the crude protein of wheat bran diets were higher than that of the basal diet. Now, we have examined the mechanism of urinary Hg excretion by wheat bran.

1571 Histopathological Examination of Kidney, Liver and Spleen of Male Sprague-dawley Rats Orally Exposed to Ras Sindoor

N. Sadekar and L. D. Trombetta. *PHS, St. John's University, Queens, NY.*

Ras Sindoor (RS) is an ayurvedic medicine commonly used in the Indian sub-continent. It is manufactured by combining purified forms of both mercury and sulfur to produce red mercuric sulfide (HgS). This mineral based medicine is indicated in a multitude of medical conditions pertaining to respiratory, cardiovascular, urogenital systems, etc. The scientific research required to establish RS's toxicological profile is missing. This has resulted in its unregulated distribution and use. An *in vivo* study was conducted for 60 days using 8-week old male Sprague-Dawley rats. Animals were divided into 5% fat diet and 35% fat diet group. Each of the diet groups consisted of control, suggested therapeutic dose of 1.8mg/kg RS and high dose of 18mg/kg RS with 3 animals per treatment group. The animals were orally gavaged with a mixture of RS and 30-40%w/w honey in water (vehicle) for treatment groups and with the vehicle alone for controls. The animals were sacrificed and various organs were fixed in 10% neutral buffered formalin or snap frozen in liquid nitrogen. Histological analysis of formalin fixed renal tissue was performed with Sevier-Munger silver stain and Phosphotungstic Acid-Hematoxylin (PTAH) stain. The silver stain sections revealed renal congestion at the 18m/kg for both diet groups as compared to controls. PTAH stain showed urinary casts in renal tubules and thickened basement membrane in glomeruli in both treatment groups when compared with respective controls. Hematoxylin-eosin stained liver sections showed tissue congestion in both diet groups in the 1.8mg/kg and 18mg/kg samples when compared to respective controls. Immunostain with Fluorescein labeled Peanut Agglutinin was performed on formalin fixed spleen tissue sections and a dose dependent increase in the activation of germinal centers was seen in 5% fat diet group as compared to control. The study outlines renal and liver tissue pathology and an induction of inflammatory response observed in spleen as a result of sub-chronic exposure to RS.

1572 The Tibetan Medicine Zuotai Influences Clock Gene Expressions in the Liver of Mice

H. Li[2], W.-K. Li[2], Q. Wu[2], L.-X. Wei[1] and J. Liu[2]. [1]*Northwest Plateau Institute of Biology of Chinese Academy of Sciences, Xining, China and* [2]*Zunyi Medical College, Zunyi, China.*

The circadian timing system rhythmically controls behavior, physiology, cellular proliferation and xenobiotic metabolism over the 24-h period. Circadian rhythm is also involved in drug metabolism, efficacy and toxicity. Drugs and toxicants could in turn affect the biological clock as a mechanism of their actions. Zuotai is an essential component of many popular Tibetan medicines for sedation, tranquil and "detoxification". Zuotai is mainly composed of metacinnabar (beta-HgS) and the pharmacological basis of its action is largely unknown. This study aimed to examine the effect of Zuotai on biological clock in the liver of mice. Mice were orally given Zuotai (10 mg/kg, 1.5-fold of clinical dose) daily for 7 days, and livers were collected every 4 h during the 24 h period. Total RNA was extracted and subjected to real-time RT-PCR analysis of circadian clock gene expression. Zuotai decreased the oscillation amplitude of the clock core gene Clock and Bmal1 (Brain and muscle Arnt-like protein-1). For the clock feedback negative control genes, Zuotai decreased the oscillation amplitude of the clock gene Cry1 (Cryptochrome circadian clock 1), but had no effect on the oscillation of the clock gene Per1-3 (Period circadian clock 1-3). For the clock-driven target genes, Zuotai decreased the oscillation amplitude of the PAR-bZip family member Dbp (D-box-binding protein), but had little influence on the nuclear receptor Rev-Erbα (NR1D1). Taken together, the Tibetan medicine Zuotai could influence the expression of clock genes, which could be one of the pharmacological and toxicological actions of Zuotai in traditional Tibetan medicines.

1573 Transient Receptor Potential A1 Contributes to Late-Stage Methylmercury Toxicity in Mouse Dorsal Root Ganglia

E. Formiller, H. Hannon and W. D. Atchison. *Michigan State University, East Lansing, MI.*

Methylmercury (MeHg) neurotoxicity disrupts sensory function including that of dorsal root ganglia (DRG). Large fiber DRG are uniquely prone to MeHg toxicity; small fiber DRG are relatively spared. Mechanisms responsible for this are unknown. Acute MeHg exposure causes a kinetically-distinct multiphasic increase in [Ca2+]i in multiple neuronal models. The 1st effect (P1) is due to of Ca2+i release; a later phase (P2) is due to Ca2+e influx. We previously showed that Ca2+e removal improves DRG viability 4 hrs following MeHg exposure. We now examined the role of transient receptor potential A1 (TRPA1), a Ca2+-permeable ion channel highly expressed in DRG, in mediating MeHg-induced cytotoxicity and Ca2+e entry. DRG from spinal levels T11-L6 were isolated from 5-8 week-old C57BL/6J mice, enzymatically and mechanically dissociated, and plated as heterogeneous populations. DRG were treated with MeHg (0.2 μM-2μM) and viability was assessed. The TRPA1 antagonist A-967079 improved DRG viability (0.5 μM MeHg: 97.5±1.6%; mean ± SEM), at all [MeHg] compared to 1.8 mM Ca2+e (53.6±10.1%). Similar results occurred in Ca2+e-free conditions (97.0±2.9%), suggesting that TRPA1-mediated Ca2+e influx contributes to late-stage cytotoxicity. Relative changes in [Ca2+]i throughout 0.5 μM MeHg exposure were assessed using Fura-2 AM. Removal of Ca2+e delayed the time-to-onset of P2 (40.4±5.3 min) compared to 1.8 mM Ca2+e (19.0±2.4 min), suggesting that Ca2+e influx is responsible for increasing [Ca2+]i during P2 of MeHg exposure. Blocking TRPA1 with A-967079 did not delay the time-to-onset of P2, suggesting it may not be a primary pathway of Ca2+ entry into the cell during MeHg exposure at the assessed timepoints. In conclusion, blocking TRPA1 improves DRG viability in late-stage MeHg toxicity. However it does not delay the time-to-onset of P2 elevations of [Ca2+]i, suggesting that TRPA1 may be responsible for large fiber DRG susceptibility, likely through means other than serving as a route of Ca2+e entry. Supported by NIH grants R01ES024064, R01ES03299 and R25ES025060.

1574 Role of Homo- and Heteromeric Nicotinic Acetylcholine Receptors in Mediating Cytotoxicity After Acute Methylmercury Exposure in PC12 Cells

M. Rios-Cabanillas and W. D. Atchison. *Department of Pharmacology/Toxicology, Michigan State University, East Lansing, MI; Environmental and Integrative Toxicology Prog and Comparative Medicine Integrative Biology Prog, Michigan State University, East Lansing, MI.*

Acute exposure to methylmercury (MeHg) disrupts internal Ca2+ regulation, potentially causing cell death. The receptors involved during the MeHg-induced increase in internal [Ca2+] ([Ca2+]i) and subsequent cell death are not yet completely identified. nAChRs appear to contribute to MeHg-induced cytotoxicity in differentiated sympathetic-neuron like PC12 (dPC12) cells. Application of the nonspecific nAChR antagonist, mecamyllamine (MEC), increases cell viability after acute MeHg exposure. As different nAChR subtypes act in cell-type specific manner, and have differential Ca2+ affinities, we compared the role of the highly Ca2+ permeable homomeric α7 subtype nAChR with other heteromeric nAChRs, to the contribution to MeHg-induced cytotoxicity. Cell viability after MeHg exposure was indicated by the fluorescent dyes: calcein AM and ethidium homodimer-1. dPC12 cells were exposed to 1, 2 or 5 μM MeHg during 1 hr in the absence or presence of the specific (α7) or nonspecific blockers: MLA (5μM) or MEC (5μM), respectively. MeHg exposure for 1 hr, significantly decreased dPC12 cell viability in a concentration-dependent manner. MEC significantly protects against MeHg at 2Hg and 5 μM MeHg. Therefore, MEC protects at low and high [MeHg]. MLA treatment significantly protects against MeHg at 2 μM MeHg only. The combination of MEC + MLA is less effective than MEC alone. Thus, nAChRs contribute to MeHg-induced Ca2+-mediated neuronal death *in vitro*. Supported by NIH grant R01ES03299.

1575 Contribution of AMPA Receptors in Methylmercury-Induced Cell Death in the Motor Neuron NSC34 Cell Line

A. Colón-Rodriguez[3], R. K. Hajela[3] and W. D. Atchison[3]. [1]*Comparative Medicine and Integrative Biology Program, Michigan State University, East Lansing, MI;* [2]*Institute for Integrative Toxicology, Michigan State University, East Lansing, MI and* [3]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Methylmercury (MeHg) leads to cell death of motor neurons (MNs) through Ca2+ mediated pathways *in vitro* and *in vivo*. Ca2+ permeable glutamate receptors α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR) are thought to contribute to these alterations. Although AMPAR are not normally permeable to Ca2+, changes in the expression of the receptor GluA2 subunit or its RNA editing enzyme, adenosine deaminase acting on RNA2 (ADAR2), lead to Ca2+ permeability. To examine the effects of *in vitro* MeHg exposure on mRNA levels of the AMPAR and the ADAR2 enzyme, a MN cell line (NSC34) was exposed to 0, 0.5, 1, and 1.5μM MeHg starting on DIV 3 and continuing for 24 or 48h. MeHg effects on cell viability were examined by Trypan blue exclusion assay. QPCR was performed on reverse transcript (cDNA) of RNA isolated

from NSC34 cells. AMPAR GluR1, 2, 3, and 4 subunits, and the ADAR2 enzyme subunit expression was measured from treated and untreated NSC34 cells. We hypothesized that MeHg would differentially affect the receptor subunits studied, decreasing expression of GluA2 and ADAR2 and increasing expression of the other subunits studied, compensating for MeHg effects and leading to the observed Ca2+ permeable AMPAR. Time and concentration-dependent cell death of NSC34 cells occur after 24 or 48h MeHg exposure. AMPAR subunits and ADAR2 levels increased after 24h exposure to 0.5μM MeHg. However, after 1 or 1.5μM MeHg exposure GluA2-4 and ADAR2 were downregulated. The mRNA levels of GluA1-4 and ADAR2 were significantly downregulated after 48h exposure to 0.5 and 1μM MeHg. This effect was similar after 1.5μM exposure for the GluA1, 2 and ADAR2 subunits. These results support the idea that MeHg effects in MNs are in part mediated by increased expression of the Ca2+ permeable AMPAR. This was mainly due to the decrease observed in the RNA editing enzyme ADAR2 and the GluA2 subunit. This work was supported by NIH grants R01ES024064 and T32ES007255-26 and by a DFI award from the MSU VPRGS Office.

1576 Spinal Cord Cell Cultures Are More Susceptible to Acute Methylmercury Exposure Than Motor Neuron Like Cells, NSC34 Cells

P. Elosegui Rodriguez[1], D. Wiwatratana[2] and W. Atchison[2]. [1]*University of Puerto Rico, Cayey, PR;* [2]*Comparative Medicine and Integrative Biology, Michigan State University, East Lansing, MI; and* [3]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI.* Sponsor: W. Atchison.

Motor neurons (MNs) are the primary targets in amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis. Previous studies from our lab demonstrated that chronic postnatal exposure to the environmental neurotoxicant methylmercury (MeHg), at doses which did not produce overt neurotoxicity, markedly enhanced development of ALS-like phenotype in a susceptible mouse model. Associated with this was dysregulation of MN [Ca2+]i homeostasis. Additionally, acute MeHg exposure altered the intracellular homeostasis in wildtype mouse MN primary cultures. As a means of studying the effects of MeHg further on MNs, we sought to characterize its effects in the NSC34 MN-like cell line to use as a MN model system. These cells are hybridomas derived from a fusion between neuroblastoma N18TG2 and mouse MN-enriched spinal cord cells. We compared the relative the susceptibility to MeHg of mouse spinal cord cell culture (SCC) and differentiated NSC34 cells. Both groups were exposed to 0, 1, 2 and 5 μM MeHg for 24h. MeHg-mediated cell toxicity was assessed using both 1) 0.4% trypan blue and 2) acridine orange (AO) and propidium iodine (PI). Both cell types had reduced viability after 24h exposure to MeHg. The SCCs were more sensitive to MeHg than NSC34 cells; at 1μM, MeHg significantly reduced SCC viability while at 5 μM MeHg significantly affected to NSC34 cells viability. Thus, while NSC34 cells are responsive to MeHg, they do not exhibit the same level of sensitivity as do primary SCCs.

1577 Mitochondrial Membrane Potential Changes in Response to Methylmercury-Induced Toxicity in Motor Neuron-Like Cells, NSC34 Cells: The Role of Dimethyl Fumarate in Neuronal Protection

L. Pérez-Castro[2], G. Bazany[1], D. Wiwatratana[4] and W. Atchison[4]. [1]*Calvin College, Grand Rapids, MI;* [2]*University of Puerto Rico in Cayey, Cayey, PR;* [3]*Comparative Medicine and Integrative Biology, Michigan State University, East Lansing, MI and* [4]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI.* Sponsor: W. Atchison.

Our previous studies indicated that chronic MeHg exposure in transgenic human Cu2+/Zn2+ superoxide dismutase1 G93A mice, and acute exposure of wildtype MNs in primary culture perturbed motor neuron (MN) intracellular calcium homeostasis . The mechanism by which MeHg induced MN toxicity is not yet clear. In this study, NSC-34 cells, a hybridoma cell line derived from neuroblastoma fusion with spinal MNs, were used as a MN cell model: 1) to compare the concentration-dependence of cytotoxicity to MeHg as compared to primary cultures of spinal cord neurons and 2) determine if the impairment of mitochondrial membrane potential (ψM) is involved in MeHg- induced MN toxicity. Cytoxicity assays with trypan blue and acridine orange and propiduim iodine indicated significant reduction of cell viability after 24h exposure to 2 and 5μM MeHg. Flow cytometry analysis of the alteration of 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) indicated a significant compromise of ψM upon exposure to 2μM MeHg, and an increase in cell death when exposed to 5μM MeHg. This correlation between Dψm and cell viability after exposure to MeHg implied susceptibility of MNs to MeHg partially due to MeHg impairment of ψM, perhaps leading to oxidative stress and cell death. We further investigated if the cytoprotective agent dimethyl fumarate (DMF) could protect NSC-34 cells from MeHg-induced cell death. Combined treatment with 10μM DMF and 2μM MeHg did not protect NSC-34 cells.

1578 Contributions of Extracellular and Intracellular Calcium Concentration in MeHg-Induced Cytotoxicity in Mouse Primary Cortical Astrocytes

J. Ortiz-Rodriguez[1], R. Jaiman-Cruz[3] and W. D. Atchison[3]. [1]*Univ of Puerto Rico-Cayey, Cayey, PR;* [2]*Dept Pharmacology/Toxicology, Michigan State University, E. Lansing, MI and* [3]*Neuroscience Program, Michigan State University, E. Lansing, MI.*

Methylmercury (MeHg) preferentially affects granule cells in the cerebellum; however neighboring astrocytes are also targeted. This study aims to compare the immediate and delayed effect of MeHg exposure on cerebellar astrocytes and the relationship between MeHg-induced disruption of intracellular and extracellular Ca2+ with cell death. We hypothesized that the incidence of cell death will be increased after a delayed exposure to MeHg, and this effect will be caused by increased levels of intracellular and extracellular Ca2+ concentrations. Primary astrocyte cultures from the cerebellum of mice were exposed for 3h to 0, 1, 2, or 5μM MeHg. Cytotoxicity was measured either immediately after the 3h exposure, or 24h later using ethidium homo-dimer and calcein-AM. To determine if astrocytes death was caused by an increase in intracellular Ca2+, the chelator BAPTA was added to chelate Ca2+ released by MeHg. In order to determine if astrocytes death was caused by the extracellular Ca2+ that entered the cell, the cell-impermeant chelator EGTA was used. Although immediate exposure to MeHg resulted in a significant % of astrocyte death, the response was more pronounced 24h after exposure, with 2 and 5 μM MeHg producing respectively 39% and 40% more cell death than the immediate exposure group. When treated with BAPTA+MeHg, there was 56% less cell death at both 2 and 5 μM MeHg when compared with the 24h after MeHg exposure group. EGTA also caused a significant decrease in cell death, of 68% and 88% at 2 and 5μM MeHg respectively. Thus both, intracellular and extracellular Ca2+ contributes to delayed cytotoxicity of cerebellar astrocytes during MeHg exposure. Supported by NSF grant DBI-1359302, and NIH grants R01ES024064 and T32GM092715.

1579 Low *In Situ* Expression of Antioxidative Enzymes in Cerebellar Granular Cells Susceptible to Methylmercury in a Rat Model of Minamata Disease

M. Fujimura[2] and F. Usuki[2]. [1]*Basic Medical Science, National Institute for Minamata Disease, Minamata, Japan and* [2]*Clinical Medicine, National Institute for Minamata Disease, Minamata, Japan.* Sponsor: A. Naganuma.

Methylmercury (MeHg), an environmental neurotoxicant, induces site-specific toxicity in the brain. Although oxidative stress has been demonstrated with MeHg toxicity, the site-specific toxicity is not completely understood. Among the cerebellar neurons, cerebellar granule cells (CGCs) appear vulnerable to MeHg, whereas Purkinje cells and molecular layer neurons are resistant. Here, we established a MeHg-intoxicated rat model to investigate these cerebellar neurons for the different causes of susceptibility to MeHg. Rats were exposed to 20 ppm MeHg for 4 weeks, and subsequently exhibited neuropathological changes in the cerebellum that were similar to those observed in humans. We first isolated the 3 cerebellar neuron types using a microdissection system, and then performed real-time PCR analyses for antioxidative enzymes. We observed that expression of manganese-superoxide dismutase (Mn-SOD), glutathione peroxidase 1 (GPx1), and thioredoxin reductase 1 (TRxR1) was significantly higher in Purkinje cells and molecular layer neurons than in CGCs in non-treated control group. MeHg exposure increased the expression level of Mn-SOD in all cerebellar neuron types of rats. Contrastingly, the expression level of GPx1 was decreased by MeHg exposure in all cerebellar neuron types. Finally, we performed immunohistochemical analyses on the cerebellum. Immunohistochemistry showed increased expression of Mn-SOD, GPx1, and TRxR1 in Purkinje cells and molecular layer neurons, which was coincident with the mRNA expression patterns. Considering Mn-SOD, GPx1, and TRxR1 are critical for protecting cells against MeHg-intoxication, the results indicate that low expression of these antioxidative enzymes increases CGCs vulnerability to MeHg toxicity.

1580 Methyl Mercury (MeHg) Alters Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Gene Expression in Primary Astrocytes

M. Culbreth and M. Aschner. *Albert Einstein College of Medicine, Bronx, NY.*

MeHg is a known developmental neurotoxicant. Despite extensive research, the mechanism(s) by which it elicits toxicity have not been fully elucidated. It has been demonstrated that MeHg induces an oxidative stress response in the brain, which in part is coordinated by Nrf2. Nrf2 is a transcription factor that translocates to the nucleus in response to reactive oxygen species generation and upregulates phase II detoxifying enzymes. It is well established that Nrf2 knockout models are more susceptible to toxicity. While these experiments have provided valuable insight, it is not surprising that Nrf2 knockout increases model vulnerability. Thus, we investigated whether MeHg had a more direct effect on Nrf2 activity. We first examined Nrf2 gene expression subsequent to MeHg exposure in astrocytes, the primary site of MeHg accumulation in the brain. Astrocytes were isolated from post-natal day 1 Sprague-Dawley rats, and exposed to MeHg (0, 0.5, 1, 5 μM) for 1, 6, or 24 hours (hr); RNA was isolated, and qRT-PCR performed. We observed a significant decrease in Nrf2 gene expression at 5 μM MeHg 6 hr (0.843 +/- 0.04, $p < 0.05$) and 24 hr (0.604 +/- 0.16, $p < 0.05$) post-exposure. A concomitant decrease in cell viability was detected at both time-points (6 hr: 76.7 +/- 11.6, $p < 0.05$; 24 hr: 38.1+/- 9.9, $p < 0.05$). We also examined Keap1 gene expression. Keap1 retains Nrf2 in the cytoplasm in the absence of oxidative stress. There was a significant decrease in Keap1 gene expression at 5 μM MeHg 6 hr (0.579 +/- 0.09, $p < 0.05$) post-exposure. These results provide evidence that MeHg could be eliciting toxicity by down-regulating Nrf2 gene expression. They also support the possibility that MeHg functions upstream of Nrf2 in modulating the oxidative stress response. (supported by NIEHS R01ES020852)

1581 Antioxidant Response During Methylmercury Neurotoxicity: Sex-Related Differences

J. A. Ruszkiewicz and M. Aschner. *Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.*

Mercury is one of the most common environmental contaminant that has been shown to cause long-lasting neurological deficits. The organic form - methylmercury (MeHg), represents the major concern because of its propensity to readily enter into the brain, especially during early development. Importantly, young male individuals appear to be less resistant than females, however the reasons for the differential susceptibility remain unclear. Disturbances in redox homeostasis are considered to be a major event in MeHg-induced neurotoxicity and are believed to occur as a result of brain mercury accumulation, followed by interaction and oxidation of thiol and selenol groups in numerous biomolecules. Endogenous antioxidants, such as components of the thioredoxin system (Trx/TrxR) represent such molecules. Disrupted activity of the thioredoxin system was reported in some *in vitro* and *in vivo* models of mercury neurotoxicity, however differences in antioxidant capacity between male and female have yet to be experimentally evaluated. We exposed mice to MeHg by placenta and by breast-feeding, reflecting physiological routes of MeHg intoxication. We measured the activity of thioredoxin (Trx) and thioredoxin reductase (TrxR) in cerebrum isolated from pups at postnatal day 21. Gene expression of the individual components of the thioredoxin system was also evaluated. Thioredoxin reductase activity was significantly decreased both in nuclear and cytoplasmic extracts from MeHg-exposed male pups, whereas in female pups there was an increase in cytoplasmic and no change in nuclear activity. Thioredoxin activity was unchanged in both groups. qRT-PCR analysis showed decreased expression of thioredoxin reductases: TrxR1, TrxR2, both in male and female mice exposed to MeHg, and decreased expression of thioredoxin Trx2 in females. These results indicate that differential regulation of gene expression is not responsible for the differences in thioredoxin reductase activity. Further studies are required to identify other possible mechanisms. Nonetheless, our findings suggest that sex-related differences in antioxidant response, particularly thioredoxin system, might contribute to a sex-dependent susceptibility to MeHg. Supported by NIEHS R01 ES07331.

1582 Role of Cystathionine Gamma-Lyase in the Health Risks of Methylmercury

M. Akiyama[1], T. Toyama[3], E. Yoshida[2], T. Unoki[1], Y. Abiko[1], Y. Shinkai[1] and Y. Kumagai[1]. *[1]Faculty of Medicine, University of Tsukuba, Tsukuba, Japan; [2]Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda, Japan and [3]Graduate School of Pharmaceutical Sciences & Faculty of Pharmaceutical Sciences, Tohoku University, Sendai, Japan.*

Methylmercury (MeHg) is an environmental electrophile that readily modifies protein thiols, resulting in dysfunction of enzymes and neurotoxicity. Endogenous reactive sulfur species (RSS) such as glutathione persulfide (GSSH) and protein-bound RSS are captured by MeHg to form bismuthylmercury sulfide (MeHg) 2S identified as a metabolite of MeHg *in vivo* (Yoshida E et al. Chem Res Toxicol 2011; Abiko Y et al. Chem Res Toxicol 2015). We also found that cystathionine gamma-lyase (CSE) is an enzyme to produce cysteine persulfide that easily reacts with GSH to yield GSSH and protein-bound RSS (Ida T et al. PNAS 2014). These findings suggest that CSE to form RSS is a key protein to diminish MeHg toxicity. To address this issue, we used CSE-KO mice: When MeHg (10 mg/kg) was intraperitoneally injected into wild-type and CSE-KO mice, (MeHg)2S was detected in the brain, heart, liver and kidney, although little appreciable formation of the metabolite was seen, suggesting that CSE plays a role in (MeHg) 2S formation *in vivo*. Exposure of wild-type mice to MeHg (5 mg/kg/day for 12 days) did not cause a toxic effect on the mice; however, CSE deletion resulted in motor dysfunction and body weight loss. These results suggest that RSS produced by CSE is essential for reduction of MeHg toxicity *in vivo*. Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (#25220103 to YK).

1583 Shining Synchrotron Light on Minamata Disease: An Infamous Organo-Mercury Poisoning Event

A. K. James[1], K. Eto[4], M. Marumoto[4], M. Sakamoto[4], J. Cotelesage[1], G. E. Watson[3], J. L. O'Donoghue[3], G. J. Myers[3], P. H. Krone[1], I. J. Pickering[1] and G. N. George[1]. *[1]Anatomy and Cell Biology, University of Saskatchewan, Saskatoon, SK, Canada; [2]Geology, University of Saskatchewan, Saskatoon, SK, Canada; [3]Medical Centre, University of Rochester, Rochester, NY and [4]Ministry of the Environment, National Institute for Minamata Disease, Kumamoto, Japan; [5]Toxicology, University of Saskatchewan, Saskatoon, SK, Canada.*

Almost 60 years ago, Japan experienced a devastating poisoning event that became known worldwide as Minamata Disease; a tragedy caused by long-term release of industrial pollution into Minamata Bay. It has been hypothesized that mercury species became methylated in Minamata Bay and subsequently biomagnified up the food chain to fish residing within the bay. These organo-mercury containing fish were eaten by the local populations, the neurologic effects of which were seen in children and adults as well as in congenital cases. The dynamics of organic mercury in the human brain are still not well understood, and the importance of chemical speciation when investigating mercury toxicity has been emphasized recently. This research has utilized X-ray absorption spectroscopy (XAS) to better understand the chemical fate of mercury in human brain tissue. This study focuses on two sufferers of Minamata Disease, one patient who passed away quickly after exposure and another patient who survived many years post-Minamata. Included in the study are two brain tissue samples per patient (cerebellum and occipital lobe tissue samples) for a total of four human brain samples. A cerebellum tissue sample from a cat exposed to effluent from the factory is also included. Analysis of the autopsy materials has revealed intriguing differences between the chemical forms of mercury sequestered in the brain tissue, which may ultimately help further elucidate the dynamics of mercury in the human brain following an acute exposure.

1584 Maternal Methylmercury Exposure through Rice Ingestion and Offspring Development

S. Rothenberg[1], X. Yu[2] and C. Hong[1]. *[1]Environmental Health Sciences, University of South Carolina, Columbia, SC and [2]Shanghai Key Lab for Children's Environmental Health, Xinhua Hospital Affiliated to Shanghai Jiao Tong School of Medicine, Shanghai, China.*

Objective: Rice ingestion is an important exposure pathway for methylmercury, a potent neurotoxin. However, rice does not contain the same beneficial micronutrients as fish (e.g., n-3 fatty acids). The objective was to determine the impacts of maternal methylmercury exposure through rice ingestion on offspring neurodevelopment. Methods:

From 2013-2014, 398 pregnant mothers were recruited at parturition from a rural hospital in Guangxi province, China. Prenatal methylmercury exposure was determined by analysis of maternal hair and blood mercury. The primary outcome measure was children's cognitive and psychomotor abilities, assessed at 12 months using the Bayley Scales of Infant Development-II (n=318 children assessed). Results: Most mothers (87%) ingested rice daily, while fish/shellfish consumption varied: never 43%, monthly 35%, weekly 20%, daily 2%. Average hair mercury (0.48 µg/g, geometric mean: 0.41 µg/g, range: 0.26-1.7 µg/g) was similar to values reported for cohorts of U.S. pregnant women. Linear regression models (unadjusted at this time) were developed including the developmental score (dependent variable) and prenatal methylmercury exposure (independent variable) for all mothers (n=318), for mothers who consumed rice daily (n=110), and for mothers who consumed rice daily and fish weekly or daily (n=59). For the mental development index (MDI), for all mothers [Beta = -1.7, 95%CI = (-6.1, 2.8)], for mothers who consumed rice daily [Beta = 0.88, 95%CI = (-6.1, 7.9)], and for mothers who consumed rice daily and fish [Beta = 1.3, 95%CI = (-9.1, 12)]. For the psychomotor development index (PDI), for all mothers [Beta = -4.1, 95%CI = (-9.1, 0.98)], for mothers who consumed rice daily [Beta = -1.0, 95%CI = (-9.0, 7.0)], and for mothers who consumed rice daily and fish [Beta = -3.5, 95%CI = (-14, 6.7)]. Conclusions: Rice ingestion was an important source of dietary methylmercury among most mothers. Neurodevelopment indices (MDI and PDI) were inversely associated with prenatal methylmercury exposure; however trends were non-significant. For mothers who ingested fish weekly or daily, the MDI slope was slightly higher (Beta=1.3) compared to mothers who did not ingest fish (Beta=0.88). However, the opposite was true for PDI (no fish, Beta = -1.0; fish, Beta = -3.5).

1585 Neuroantibodies (NAb) in Males with Mercury (Hg) Exposure: Prevalence and Association with Clinical Signs

H. A. El-Fawal[3], I. Abdel Moneim[1,2], R. M. El-Gazzar[2] and M. Y. Shamy[1,2]. *[1]King Abdulaziz University, Jeddah, Saudi Arabia; [2]Institute of Public Health, University of Alexandria, Alexandria, Egypt and [3]Neurotoxicology Laboratory, Albany College of Pharmacy and Health Sciences, Albany, NY.*

To further assess the use of NAb as biomarkers of nervous system insult, 65 demographically matched males (38+6yo) from two industries, food packing (n=26) and fluorescent light factories (n=39), were recruited to provide a wide range of internal dose, as determined by urinary Hg (HgU). In addition to work and medical history, a neurological exam consisting of upper and lower limb sensorimotor assessment was scored from 0-6 according to spinothalamic, dorsal column and muscle deficits. HgU was determined (median:2.1; range:0.5-26 µg/g creatinine) in spot urine and was the basis of stratification into tertiles (<1.2, n=11; 1.21-2.8, n=14; >2.81, n=40) for data analysis. Serum IgM and IgG against neurofilaments (NF-L, NF-M and NF-H), GFAP and myelin basic protein (MBP) were determined and together with HgU, years of work exposure and clinical scores stratified after an overall Spearman correlation. Anti-GFAP and anti-MBP, IgM, and anti-NF-L, NF-M and NF-H, IgG, significantly associated (r>0.35; p<0.02) with HgU, years of exposure and clinical scores, but not age or smoking. Prevalence (percent with titers) of anti-GFAP and anti-MBP, IgM, and anti-NF-L, NF-M and NF-H, IgG, as well as clinical scores were significantly (p<0.05) higher in the third tertile compared to the lower two tertiles, which did not differ from each other. Nonparametric comparison of NAb titers between tertiles consistently evidenced higher (p<0.0001-0.05) levels of anti-GFAP and anti-MBP, IgM, and anti-NF-L and NF-H, IgG, as well as higher clinical scores for males in the high HgU tertile. Multiregression modeling suggested that HgU and years of exposure were the primary determinants of NAb titers and of clinical scores (r2>0.2; p=0.0001-0.05), while IgG for NF-H and IgM for GFAP were significant determinants of clinical scores. The persistent pattern of higher prevalence, titers and strong associations with exposure measures for anti-NF, IgG, the isotype associated with immunological memory and acute insult, and anti-GFAP and anti-MBP, IgM, the isotype associated with chronic insult and primary challenge, suggests neuronal involvement preceding glial insult. This study further validates the use of NAb in monitoring individuals at risk of environmental neurotoxicity.

1586 Copper Exposure Alters Brain Inflammatory Responses and Impairs Clearance of Amyloid-beta

M. Kitazawa and H.-W. Hsu. *Molecular and Cell Biology, University of California, Merced, Merced, CA.*

Chronic exposure to copper could potentiate abnormal buildup of amyloid-beta (Aβ) and development of neurofibrillary tangle pathology in animal models of Alzheimer's disease (AD). However, the exact molecular mechanism by which copper exposure increases the risk of developing AD-like neuropathology remains to be elucidated. We hypothesized that the neurotoxic effect of copper was in part mediated by the potentiation of microglial inflammatory responses in the brain, which lead to an impairment of Aβ clearance. To test this hypothesis, we examined the effect of copper on inflammation and Aβ clearance mechanisms using both *in vitro* and *in vivo* models of the disease. When murine monocyte BV2 cells was exposed to copper, Aβ-elicited cytokine production was exacerbated, and the ability of BV2 cells to effectively phagocytose fibrillar Aβ was significantly reduced. Interestingly, we also found that pro-inflammatory cytokines, IL-1β, IL-6 and TNFα, markedly reduced the steady-state levels of low-density lipoprotein receptor-related protein 1 (LRP1) in human microvascular endothelial cells (MVECs) in a concentration-dependent manner. These findings suggest that the two major Aβ clearance mechanisms, Aβ phagocytosis and LRP1-dependent Aβ transport, in the brain were impaired following the exposure to copper. These changes were also detected in mouse models of AD chronically exposed to copper-containing drinking water. Taken together, our findings support the pathological impact of copper on inflammatory responses and Aβ clearance in the brain, which could serve as a key mechanism to explain the chronic copper exposure as an increasing the risk for developing AD.

1587 Roles of Lead Exposure in Alzheimer'S Disease: Relationship to Brain Amyloid Aggregation

Y. Du[2,3], P. Territo[2] and W. Zheng[3]. *[1]Neurology, Indiana University School of Medicine, Indianapolis, IN; [2]Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN and [3]School of Health Sciences, Purdue University, West Lafayette, IN.*

Accumulation of beta-amyloid (Abeta), particularly its oligomer, in brain is believed to be a hall mark of Alzheimer's disease (AD) pathogenesis. Recent human epidemiological evidences suggest an association between human lead (Pb) exposure and altered gene expressions relevant to the Abeta production. Our early studies also show a reduced Abeta clearance from the Pb-exposed brain, contributing to the Abeta buildup in brain. In the current study, we used human Tg-SWDI APP transgenic mice, which genetically over-express amyloid plaques at age of 2-3 months, to investigate if Pb exposure caused the amyloid oligomer accumulation in brain. Immunocytochemical analysis showed that subchronic oral Pb exposure (50 mg/kg Pb acetate for 4 weeks) increased levels of Abeta oligomers in mouse brain. Since Abeta in blood is a potential source of brain Abeta, we used the dynamic contrast-enhanced computed tomography (DCE-CT) to investigate if Pb exposure changed the BBB permeability in these transgenic mice. The imaging data showed that there was a clear Pb-induced spatial reduction in tissue perfusion, permeability-surface area, and fractional blood volume when compared with sham-treated, age-matched controls, suggesting the BBB permeability was significantly increased in Pb-treated animals. These data indicate that subchronic Pb exposure damages the blood-brain barrier structure, which in turn facilitates the diffusion of blood-borne Abeta into brain parenchyma. Together with its direct toxicity on Abeta clearance, Pb exposure increases amyloid aggregation; this may lead to Pb-associated AD. (Supported in part by NIH Grant ES008146)

1588 Embryonic Exposure to 100 µG/l Lead Results in Expression Changes in Genes Associated with Nervous System Development and Function in the Aged Adult Zebrafish Brain with Sex-Specific Alteration in P38 Mapk

J. Lee and J. L. Freeman. *School of Health Sciences, Purdue University, West Lafayette, IN.*

A developmental lead (Pb) exposure has been proposed as an environmental risk factor for adult neurodegenerative diseases including Alzheimer's disease (AD). Recent animal studies showed pathological characteristics of AD in adults with a developmental Pb exposure. However, in a recent study we did not observe gene expression alterations in major genetic risk factors for AD in brains of aged adult ze-

brafish with an embryonic Pb exposure. To further investigate the relationship between an embryonic Pb exposure and latent neurological alterations, the brain of adult male and female zebrafish aged 12 months that were exposed to a control treatment or 100 µg/L Pb only during embryogenesis (1- 72 hours after fertilization) were analyzed on a zebrafish-specific microarray platform. Gene ontology and pathway analysis revealed alterations in distinct sets of genes associated with nervous system development and function in each sex. In males, expression alterations of several genes were found to be associated with abnormal morphology of vertebrae, action potential of neurons, and evoked potential. On the other hand, in females, significant changes in expression profiles occurred in a number of genes involved in development of central nervous system (CNS), abnormal morphology of CNS/brain, and also quantity of neurons. In addition, the microarray data indicated a sex-specific involvement of p38 MAP Kinase (MAPK) as an upstream molecule involved in cell signaling pathways (e.g., neuronal apoptosis). Western blot analysis confirmed increased expression of p38 MAPK protein in aged male zebrafish only. Overall, these results suggest disturbances of global gene expression patterns by an embryonic Pb exposure supporting neurological alterations in later life. With these findings, future studies on mechanisms underlying the observed gene expression changes will facilitate our understanding of the impact of a developmental Pb exposure on neurological disease pathogenesis.

PS 1589 Role of Synaptic Structural Plasticity in Impairments of Spatial Learning and Memory Induced by Developmental Lead Exposure in Wistar Rats

Y. Xiao[1], H. Fu[2], X. Han[1], X. Hu[1], Y. Chen[1], Q. Wei[1] and Q. Hu[1]. *[1]Faculty of Preventive Medicine, School of Public Health,Sun Yat-sen University, Guangzhou, China and [2]The Jackson Laboratory, Bar Harbor, ME.* Sponsor: Y. He.

Lead (Pb) is found to impair cognitive function. Synaptic structural plasticity is considered to be the physiological basis of synaptic functional plasticity and has been recently found to play important roles in learning and memory. To study the effect of Pb on spatial learning and memory at different developmental stages, and its relationship with alterations of synaptic structural plasticity, postnatal rats were randomly divided into three groups: Control; Pre-weaning Pb (Parents were exposed to 2 mM PbCl2 3 weeks before mating until weaning of pups); Postweaning Pb (Weaned pups were exposed to 2 mM PbCl2 for 9 weeks). The spatial learning and memory of rats was measured by Morris water maze (MWM) on PND 85~90. Rat pups in Pre-weaning Pb and Post-weaning Pb groups performed significantly worse than those in Control group ($p<0.05$). However, there was no significant difference in the performance of MWM between the two Pb-exposure groups. Before MWM (PND 84), the number of neurons and synapses significantly decreased in Pre-weaning Pb group, but not in Post-weaning Pb group. After MWM (PND 91), the number of synapses in Pre-weaning Pb group increased significantly, but it was still less than that of Control group ($p<0.05$); the number of synapses in Post-weaning Pb group was also less than that of Control group ($p<0.05$), although the number of synapses has no differences between Post-weaning Pb and Control groups before MWM. In both Pre-weaning Pb and Post-weaning Pb groups, synaptic structural parameters such as thickness of postsynaptic density (PSD), length of synaptic active zone and synaptic curvature increased significantly while width of synaptic cleft decreased significantly compared to Control group ($p<0.05$). Our data demonstrated that both early and late developmental Pb exposure impaired spatial learning and memory as well as synaptic structural plasticity in Wistar rats.

PS 1590 The TrkB Receptor Ligand 7,8-dihydroxyflavone (7,8-DHF) Reverses the Effects of Developmental Lead Exposure on Presynaptic Neurotransmitter Release in Adult Rat Hippocampus

P. K. Stanton[1], X.-I. Zhang[1], K. H. Stansfield[2], J. L. McGlothan[2] and T. R. Guilarte[2]. *[1]Cell Biology & Anatomy, New York Medical College, Valhalla, NY and [2]Environmental Health Sciences, Columbia University, New York, NY.*

Lead exposure during brain development inhibits neurotransmitter release and this effect is likely to contribute to impaired synapse formation, plasticity and learning deficits. In dissociated primary hippocampal neurons in culture (Neal et al., Toxicol Sci, 116:249-63, 2010) and hippocampal slices (Zhang et al., PLoS ONE, 10(5):e0127461, 2014), lead exposure inhibits vesicular release and reduces the number of fast-releasing sites, an effect mediated in cultures by lead inhibition of NMDA receptor-mediated transsynaptic BDNF signaling. Therefore, we hypothesized that activation of TrkB receptors, the cognate receptor for BDNF, might restore the lead-induced impairment of presynaptic function. We report that daily post-weaning injection of the TrkB agonist 7,8-DHF (5 mg/kg) during the last 14 days of lead exposure reversed the lead-induced impairment of presynaptic transmitter release at Schaffer collateral-CA1 synapses in *ex vivo* hippocampal slices. Chronic dietary lead exposure (1500 ppm; blood lead levels 21.1 +/- 1.6 µg/dL; n=15) significantly enhanced paired-pulse facilitation (PPF) [Control = 1.7; Lead PPF = 3.0 at 30msec inter-pulse interval], an indirect measure of reduced release probability. Mean-variance analysis of evoked synaptic potentials at Schaffer collateral-CA1 synapses directly confirmed that lead exposure persistently reduced transmitter release probability (Control Pr = 0.46; Lead Pr = 0.25 at 2mM [Ca2+]o). Using FM1-43 2-photon imaging of release from CA1 Schaffer collateral terminals, we showed that this reduced probability was associated with reduced release of glutamate from vesicles in the rapidly-recycling vesicle pool loaded by hypertonic shock. All of these effects of perinatal lead were rescued by co-administration daily of 7,8-DHF. These studies show for the first time that 7,8-DHF activation of TrkB receptors is able to reverse the effects of lead exposure on presynaptic terminals, and point to TrkB receptors as a potential target for therapeutic intervention in lead-intoxicated children. [Supported by NIH grant # ES020465]

PS 1591 The Olfactory Tubercle: A Novel Target for Lead-Induced Cocaine Responsivity

K. H. Stansfield, B. D. Soares, J. L. McGlothan and T. R. Guilarte. *Environmental Health Sciences, Columbia University, New York City, NY.*

The dopaminergic system (DAS) underlies many processes affected by lead exposure (Pb2+), such as attention, reward, and addiction. Previous studies indicate that lead (Pb2+) exposure sensitizes animals to addictive drugs, but the mechanism(s) underlying this effect have not been elucidated. Recently, our laboratory demonstrated increased cocaine-induced locomotor activity in adult rats after Pb2+ exposure. Administration of the D1 dopamine receptor (D1R) antagonist SCH-23390 blocked this effect, indicating that the stimulatory effects of cocaine in Pb2+-treated rats may be mediated by D1R. In the present study, we investigated the impact of chronic Pb2+ exposure on the DAS of juvenile (PN14), adolescent (PN28) and adult (PN50) male rats in brain regions implicated in drug addiction. We measured D1R and D2-dopamine receptor (D2R) levels in the striatum (STR), nucleus accumbens (NAC), and olfactory tubercle (OT) using quantitative receptor autoradiography. Following exposure to 1500ppm Pb2+-acetate diet, D1R expression increased in the OT (48% at PN28), NAC (21% at PN28), and STR (22% at PN28) as well as increases in D2R in the STR (30% at PN14). Following exposure to 180ppm Pb2+-acetate diet, D1R expression increased only in the OT (16% at PN28). This study is the first to demonstrate upregulation of D1R in the OT following Pb2+ exposure. Furthermore, our results show that D1R levels in the OT are markedly elevated by Pb2+ exposure relative to the STR and NAC, even at blood lead levels as low as 5 µg/dL. Studies by Ikemoto et al have demonstrated that the OT is more sensitive to the psychostimulant effects of cocaine than the STR and NAC. Our results suggest that Pb2+ exposure-induced D1R upregulation in the OT is a likely mechanism underlying the increased cocaine-induced locomotor activity in Pb2+-exposed rats reported by Stansfield et al (2015). [Supported by ES006189 and ES020465 to TRG]

PS 1592 Polychlorinated Biphenyls (PCBs) and Lead Act Synergistically on Brain Dopamine Cells *In Vitro*

S. H. Enayah. *Human Toxicology Program, University of Iowa, Iowa City, IA.* Sponsor: I. Fuortes.

Toxicity is usually studied one compound at a time. This is crucial for the understanding of the mechanism of action, but does not reflect the reality. Both, PCBs and/or Pb, are present in many older buildings. *In vivo* and epidemiological studies propose that the dopaminergic (DA) system is highly susceptible to lead (Pb) and PCBs, but through different mechanisms, and both compounds were associated with diseases such as Parkinson's and Alzheimer's. To our knowledge no study has analyzed the interaction between the combination of these compounds *in vitro* or *in vivo*. Our HYPOTHESIS is that co- exposure to Pb2+ and PCBs *in vitro* is neurotoxic at low concentrations which have no effect during individual exposure to either compound. We exposed dopaminergic PC-12 cells to PCB153, the major PCB congener in human bodies, PCB95, a neurotox PCB congener, or Pb, alone or in combination. Toxicity endpoints of interest are cytotoxicity (resazurin assay), intracellular reactive oxygen species (ROS, with DCFDA), dopamine levels, and gene expression levels of genes involved in dopamine metabolism and transport, i.e. tyrosine hydroxylase (TH),dopamine active transporter (DAT), and the vesicular monoamine tranporter (VMAT1). First experiments showed problems

due to low solubility of Pb acetate in our medium which could not be resolved by sonication, acidification, or the presence of serum. Use of medium without phosphate solved the problem, resulting in efficient uptake of Pb into the cells. Our data so far also show that co-exposure to PCBs and Pb lowers the concentration threshold where cytotoxicity is seen and results in elevated ROS levels at lower concentrations and earlier time points. Dopamine and gene expression analysis is in progress to elucidate the mechanisms of this this increased toxicity. These results show that occupational co-exposure to PCBs and Pb from old buildings may be hazardous to workers at concentrations that individually are considered as safe. (Supported by NIEHS Iowa Superfund Program P42 ES013661)

1593 Adult-Only Lead Exposure Impairs Adult Hippocampal Neurogenesis and Cognitive Behavior in Knock-In Mice Expressing the Human Apolipoprotein E4 Allele

A. K. Engstrom[1], N. Maeda[2] and Z. Xia[1]. [1]*Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and* [2]*Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.*

Through a process called adult neurogenesis, adult neural precursor cells (aNPCs) in the dentate gyrus (DG) of the hippocampus generate adult-born neurons. These neurons functionally integrate into existing neuronal circuits and may contribute to hippocampus-dependent learning and memory. A variety of physiological and pathological stimuli have been shown to modulate adult hippocampal neurogenesis, and the disruption of this process may impair learning and memory, accelerate cognitive decline, and contribute to neurodegenerative disease. However, little is known about how gene-environment interactions (GxE) between toxicants and genetic risk factors may perturb adult hippocampal neurogenesis and accelerate cognitive decline. We previously found that the heavy metal lead inhibits adult neurogenesis *in vitro*. Based on our *in vitro* findings, we chose to determine whether there is a GxE between the heavy metal lead and the ε4 allele of the apolipoprotein E gene (APOE-ε4) - the strongest genetic risk factor for late-onset, sporadic AD. Humanized knock-in mice expressing either the human APOE-ε4 or APOE-ε3 alleles were exposed to 0.2% lead acetate via drinking water for 3 months starting at 8 weeks of age. Lead treatment significantly impaired adult hippocampal neurogenesis in APOE-ε4 mice compared to control (APOE-ε3) animals. In addition, compared to APOE-ε3 animals, lead-treated APOE-ε4 mice had significant and persistent deficits in hippocampus-dependent learning and memory, including impaired spatial memory in the novel object location test and decreased contextual fear memory. Our preliminary results suggest that a GxE between lead and APOE-ε4 may perturb adult hippocampal neurogenesis.

1594 The Toxic Effects of Cadmium on Adult Neurogenesis

H. Wang, A. K. Engstrom and Z. Xia. *Toxicology Program, Department of Environmental and Occupational Health Science, University of Washington, Seattle, WA.*

Adult neurogenesis is a process that occurs throughout life in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and in the subventricular zone (SVZ) along the lateral ventricles in mammalian brains. It plays an important role for the formation of hippocampus-dependent memory and olfaction. The effect of neurotoxicants on adult neurogenesis is just beginning to be elucidated. Cadmium (Cd) is a heavy metal with a long biological half-life in humans (from ten to thirty years) and has been recognized as one of the most toxic environmental and industrial pollutants. Cd is a neurotoxicant and can cause cognitive and olfactory impairment. However, molecular and cellular mechanisms underlying Cd neurotoxicity are not fully understood. The goal of our study is to investigate the effects of cadmium on adult neurogenesis and the potential underlying molecular mechanisms. To accomplish this goal, we prepared adult neural stem/progenitor cells (aNPSCs) isolated from the SGZ or SVZ of adult mice. We found that exposure of low concentrations of Cd decreased cell number and cell proliferation while induced apoptosis in cultured aNPSCs. Cd also inhibited the spontaneous neuronal differentiation of SVZ-derived aNPSCs. Furthermore, Cd exposure significantly increased phosphorylation of c-Jun NH2-terminal kinase (JNK), and p38 MAP kinase in SVZ cells, indicative of their activation. By using pharmacological inhibitors and SVZ-derived aNPSCs prepared from JNK3 knock-out mice, we found that the JNK and p-38 MAPK signaling pathways are critical for Cd neurotoxicity in aNPSCs. Moreover, *in vivo* Cd exposure significantly decreased the survival of adult born neurons in the SGZ of adult mice. Our data suggests that Cd exposure can impair adult neurogenesis, and that activation of the JNK and p38 MAP kinases play important roles in the neurotoxicity of Cd in aNPSCs. These results are, to our knowledge, the first demonstration that Cd impairs adult neurogenesis, providing new insights concerning molecular and cellular mechanisms of Cd neurotoxicity.

1595 A Gene-Environment Interaction Study to Understand the Role of Native Alpha-Synuclein in Cadmium-Induced Neurotoxicity

J. A. Jimenez[2], W. Chong[2], G. Kwakye[2] and M. Saito[1]. [1]*Woods Hole Oceanographic Institution, Woods Hole, MA and* [2]*Neuroscience, Oberlin College, Oberlin, OH.*

Alpha-synuclein (α-syn) is a ubiquitous protein whose native function is unknown. α-syn comprises 60% of Lewy bodies that are aggregates present in the brains of postmortem Parkinson's disease (PD) patients and implicated in the pathogenesis of dopaminergic neurons. 5-10% of PD cases are familial with the remaining being idiopathic. Long-term exposure to pesticides or metals such as cadmium (Cd) has been proposed to represent a risk factor for PD. Cd is an industrial and environmental pollutant that causes severe damage to a variety of organs, including the brain, and impairs cellular mechanisms implicated in PD. Here we investigated the role of native α-syn in Cd-induced neurotoxicity in an established α-syn dopaminergic cell model of PD that overexpresses human native α-syn (N27-syn) or empty vector (N27-vec). We demonstrate that N27-syn expressing cells exhibit a significant dose-dependent susceptibility to Cd neurotoxicity compared to N27-vec following a 24h exposure. Furthermore, we show that N27-syn cells exhibit lower levels of the antioxidant glutathione and increased production of reactive species compared to N27-vec, suggesting oxidative stress mediated cell death. As α-syn may be a protein localized to the cell membrane, we hypothesized that the protein may play an important role in modulating metal transporters. Consequently, we quantified intracellular Cd levels in N27 cells following a 6h and 24h Cd exposure with inductively-coupled plasma mass spectrometry (ICP-MS) and report that N27-syn cells accumulate significantly more Cd in a time-dependent manner compared to N27-vec cells. Together these findings suggest that α-syn enhances Cd-induced neurotoxicity resulting in oxidative stress mediated cell death by playing a role in Cd homeostasis. In conclusion, we have identified a novel gene-environment interaction between α-syn and Cd that may contribute to the understanding of the function of native α-syn and its response to heavy metals, potentially modifying PD neuropathology.

1596 Exposure to Metal Mixtures in Welding Fume: Effects on Neuropsychological Functions

E. J. Ward[2], R. Bowler[1], M. Nour[2], S. Snyder[2], F. Rosenthal[2] and U. Dydak[2,3]. [1]*San Francisco State University, San Francisco, CA;* [2]*Health Sciences, Purdue University, West Lafayette, IN and* [3]*Radiology, Indiana University School of Medicine, Indianapolis, IN.*

Welding fume is a mixture of metals and gases to which millions of welders worldwide are exposed to everyday. Contrary to most previous studies, who focused on single metal exposures, we investigated the neurological effects of exposure to Mn as well as other prevalent welding fume metals, such as, Copper (Cu), Zinc (Zn), Iron (Fe), Aluminum (Al), and Lead (Pb). Exposure of 28 welders and 18 controls was assessed using two methods. Firstly, air exposure to each metal was calculated for individuals based on their personal work history and current departmental airborne concentration levels. Secondly, human toenail metal concentrations were used, which has recently been validated as good biomarker of Mn exposure in welders, including in our own studies. On average, the welders' respirable airborne concentrations were Mn (0.12mg/m3), Zn (0.01 mg/m3), Cu (0.02 mg/m3), and Fe (1.19 mg /m3). In testing neuropsychological performance three cognitive and three motor tests were chosen. Psychomotor function was assessed with the Finger Tapping test (FTT), Grooved Pegboard test, and Parallel Lines test. Results indicate that decreases of cognitive flexibility (Rey-O Immediate recall) and executive function (Trail making Tests B), are associated with higher toenail Cu ($P < 0.01$); higher Pb ($P < 0.02$ and $P < 0.04$); and higher Al ($P < 0.05$). Verbal category fluency scores (Animal and Fruit Naming Tests) were found to decrease in individuals with increased toenail Mn ($P < 0.01$), Cu ($P < 0.001$ and $P < 0.02$), Pb ($P < 0.01$). Psychomotor speed (assessed by the Finger Tapping Test), and fine tactile dexterity (Grooved-Pegboard Test) were associated with increased toenail Mn ($P < 0.01$ and $P < 0.05$). Lastly, increased toenail Fe was associated with greater graphomotor tremor on the Parallel Lines Test ($P < 0.01$). These current results indicate that chronic exposure to any of the metals Cu, Pb, Al, Fe and Mn in welding fume metals have an effect on neurological performance. (Supported by NIEHS R01 ES020529 and CDC/NIOSH T03 OH008615)

1597 A Preliminary Study About Antidepressant-Like or Antianxiety-Like Activity of Tibetan Medicine Zuotai Containing Heavy Metal by the Tail Suspension Test(TST) and the Chronic Unpredictable Mild Stress(CUMS) Model of KM Mice

J. Zhao, Y. Du, C. Li, M. Zhang, H. Yang, H. Bi and L. Wei. *Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China.*

Zuotai is one of the most famous and important drugs in Tibetan medicine containing heavy metals. Previous research has found that Zuotai is a kind of ancient nano-like drug, and its main phase compositions contain β-HgS, S8, α-HgS etc. This shows that the chemical compositions between Zuotai and Chinese medicine Cinnabar has a certain similarity, so whether Zuotai have antidepressant-like or antianxiety-like effects as Cinnabar? So far, no reports have been found in literatures. Therefore, in the present study, two classic animal models, the Tail Suspension Test(TST) and the Chronic Unpredictable Mild Stress(CUMS) model of KM mouse, has been used to evaluate the antidepressant-like or antianxiety-like activities of Zuotai. The TST (n=10) results show that Zuotai can significantly reduce the immobility time (65.04±11.37, 58.28±9.61 and 62.76±11.23 s) of mice comparing with control group (93.29±5.39 s) at three doses (60.697, 303.487 and 606.97 mg•kg-1 •day) after 14-day treatment, respectively. The CUMS experiment results suggest that Zuotai can significantly alleviate the CUMS-induced depressive symptoms of KM mice, including the reduced sucrose consumption, the increased immobility, the generated anxiety-like behavior at doses 6.0697, 60.697 and 606.97 mg•kg-1 •day after 42 day treatment. Additionally, it is has been found that Zuotai can reverse the CUMS-induced 5-HT, NE concentration decreases in the serum and BDNF level decrease in KM mouse hippocampus, also can inhibit the increased CORT and ACTH concentrations in the serum by CUMS. In conclusion, the present research indicate that Zuotai possesses potentially antidepressant-like or antianxiety-like effects in the TST and CUMS models of KM mouse, and may exerts these treatment effects by modulating the HPA axis, monoamine neurotransmitter and BDNF expression in the hippocampus.

1598 Investigating Zinc Toxicity in Olfactory Neurons: *In Silico*, *In Vitro*, and *In Vivo* Studies

H. Hsieh[1], K. S. Vignesh[3], M. C. Horwath[3], T. M. Hopkins[3], G. S. Deepe[3], S. K. Pixley[3] and M. B. Genter[1]. [1]*Department of Environmental Health, University of Cincinnati, Cincinnati, OH;* [2]*Department of Molecular and Cellular Physiology, University of Cincinnati College of Medicine, Cincinnati, OH and* [3]*Division of Infectious Diseases, University of Cincinnati College of Medicine, Cincinnati, OH.*

Zinc is both an essential and potentially toxic metal. It is widely believed that oral zinc supplementation can reduce the effects of the common cold; however, there is strong clinical evidence that intranasal (IN) zinc gluconate (ZG) treatment for this purpose causes anosmia, or the loss of the sense of smell, in humans. Using the rat olfactory neuron cell line, Odora, we investigated the mechanism by which zinc is toxic to olfactory neurons. Following treatment of Odora cells with 100 and 200 μM ZG for 0-24 hr, we performed RNAseq and *in silico* analyses and found upregulation of pathways associated with zinc metal response, oxidative stress, and ATP production. We also observed that Odora cells could recover from zinc-induced oxidative stress, but ATP depletion persisted with longer exposure to ZG. We also found that ZG exposure caused an increase in NLRP3 and Il-1β protein levels in a time-dependent manner, suggesting that zinc causes an inflammasome-mediated cell death, pyroptosis, in olfactory neurons. The role of metallothionein (Mt), a metal response protein whose polymorphisms are associated with metal toxicity, particularly in developing nations, was also investigated. *In vitro* studies using shRNA knockdown of Mt1/2 demonstrated basal elevated level of markers of reactive oxygen species and depressed levels of reduced glutathione; however, the cells were still able to recover from excess zinc and showed a similar LC50 with untransfected Odora cells. *In vivo* studies utilizing wild-type, MT1/2 knockout mice (KO), and heterozygotes administered ZG by IN instillation showed profound loss of the olfactory mucosa in the nasal cavity. Recovery was monitored, and a lower percentage of the mice were able to smell 28 d after treatment; however, no significant difference was observed in the average thickness of the olfactory epithelium between KO and wild type mice. Both *in vitro* and *in vivo* studies showed downregulation of actinin levels, which may be the trigger for ZG-induced olfactory mucosal damage.

1599 Arsenic Induces *In Vitro* and *In Vivo* Accumulation of Alpha-Synuclein: Implications in Parkinson's Disease

A. B. Cholanians, S. S. Lau and T. J. Monks. *Pharmacology and Toxicology, The University of Arizona, Tucson, AZ.*

Parkinson's Disease (PD) is the second most common neurodegenerative disorder, the majority of cases being idiopathic. PD belongs to a larger family of diseases, collectively known as synucleinopathies, which are characterized by accumulation of alpha-synuclein (SYN). Environmental exposure to redox active compounds can lead to the development of PD, and arsenic (As)-containing pesticide use is correlated with increased incidence of PD. SYN a small neuronal protein with prion like properties, plays a central role in PD pathogenesis. SYN can misfold and generate toxic oligomers/aggregates, which can be cytotoxic. Moreover, As exposure can lead to an inhibition of autophagy. We hypothesize that As exposure could facilitate the accumulation of toxic SYN oligomers/aggregates. The current project examined the role of As in the oligomerization of SYN, and consequences thereof. Low level chronic As exposure in SH-SY5Y cells overexpressing SYN caused a dose-dependent oligomerization of SYN, with a concomitant increase in protein ubiquitination, suggesting an increased proteotoxic stress. Immunocytochemistry of SH-SY5Y cells revealed an accumulation of SYN, and co-localization of SYN with LC3, a major autophagic protein. Mice exposed to As (100 ppb) for one month, exhibited elevated SYN accumulation in the cortex and striatum, as evidenced by both immunohistochemical and Western blot analysis. As exposed mice also exhibited elevated ubiquitination and LC3 I and II levels in the cortex and striatum. However tyrosine hydroxylase (TH), a recognized indicator of dopaminergic cell density, was upregulated in the As exposed animals. Since SYN can inhibit TH function, exposure to As possibly leads to compensatory mechanisms leading to an increase in TH expression. Our findings suggest that susceptible individuals may be at higher risk of developing PD or other synucleinopathies due to chronic environmental As exposure. (P30 ES006694, T32 ES016652, T32 ES007091)

1600 Inhibition of ADAM17 Reduces EMMPRIN Expression after Mustard Exposure of Corneal Organ Cultures

M. K. Gordon[2], A. DeSantis-Rodrigues[2], Y. Chang[2], R. A. Hahn[2], P. Zhou[2], D. R. Gerecke[2] and K. K. Svoboda[1]. [1]*Biomedical Sciences, Baylor College of Dentistry, Texas A&M University, Dallas, TX and* [2]*Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

Recently a cancer cell line that expressed both ADAM17 and EMMPRIN was used to see whether these molecules influenced each other. Inhibiting EMMPRIN expression with siRNA had no effect on ADAM17 levels, but inhibiting ADAM17 with siRNA significantly inhibited EMMPRIN expression (Xu et al. Tumour Biol. 35:7575-75-86, 2014). This suggested that ADAM17 was involved in promoting EMMPRIN expression and/or activity in these cancer cells. Wondering whether this was a feature specific to the cancer cells or whether it might be a general phenomenon, we examined whether ADAM17 was activated quickly in corneas injured by nitrogen mustard (NM), and whether inhibiting ADAM17 activation had any effect on EMMPRIN expression. NM was applied to sets of rabbit corneal organ cultures for 0 min (i.e., added and immediately washed off), or 5 min, or 10 min, followed by washing off the agent and processing the corneas for ADAM17 activity using the Innozyme TACE activity assay kit. ADAM17 activity was found to be turned on immediately by NM exposure. Since ADAM17 is activated by phosphorylation via ERK, we evaluated whether inhibiting the transfer of the phosphate would reduce ADAM17 activity, and found that it did. Then, because we had identified a hydroxamate, N-octyl-N-hydroxy-2-[4-hydroxy-3-methoxyphenyl acetamide, aka NDH4417, as an inhibitor of ADAM17 (as assessed by the Innozyme TACE activity assay kit), we tested whether NM-exposed corneas treated with this hydroxamate would also show a reduction in EMMPRIN. EMMPRIN expression was, in fact, reduced 10 fold in NM-exposed corneas treated with the ADAM17-inhibiting hydroxamate. This reduction in the level of EMMPRIN resulted in reduced MMP-9 activity. In all, the inhibition of ADAM17 and MMP-9 activity, plus the reduced EMMPRIN expression, strongly suggest that NDH4417 effectively attenuates some of the most adverse effects of ocular vesicant injury, and that it should be evaluated as a potential mustard therapy.

1601 Development of an Ocular Vapor Exposure Model for Chloropicrin and Hydrofluoric Acid

R. D. Causey[1], J. J. Autrey[2], K. J. McGowan[2], H. Cheng[1], J. G. Lehman[1], J. Lakin[1] and A. L. Ruff[1]. [1]*US Army Medical Research Institute of Chemical Defense, APG, MD and* [2]*US Army Public Health Command, APG, MD.*

Toxic industrial chemicals (TICs) that are highly toxic, produced in large quantities, and are readily available present a security concern. Some TICs have already been effectively used as a form of terror, and there is a need to study the threat these chemicals present. Many TICs are known to cause acute and chronic ocular injuries. Effective therapeutics to treat these injuries do not exist, and a deeper understanding of the cellular and molecular mechanisms of these ocular injuries is necessary for rational therapeutic development. Mice have been and are widely used to study ocular injury and disease. Many of the cellular and molecular events implicated in the pathogenesis of human eye disease/injury have correlated well to mouse models. For these reasons, we have developed murine models for the study of ocular injury induced by selected TICs. Chemicals in the Chemical Terrorism Risk Assessment (CTRA) list are not assessed specifically for ocular threat. By cross-referencing the CTRA list with cases of industrial accidents on ToxNet, we selected hydrogen fluoride (HF) and chloropicrin (CP) for study. Using vapor cup exposure methodology developed from previous sulfur mustard studies, we conducted dose response studies to develop models of CP- and HF-induced ocular injury. Injury was evaluated for 12 weeks by slit-lamp microscopy and histopathology. The target injury was a moderate corneal injury characterized by acute inflammation and corneal epithelial damage, followed by a progressive corneal injury that affects visual acuity. Studies in progress will evaluate gene expression profiles in injured corneas. Future studies will evaluate injury by electron microscopy and immunohistopathology. Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for Care and use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544) as amended. This research was supported by an interagency agreement between NIH/NIAID, the USAMRICD.

1602 Endothelial Toxicity is Responsible for Acute and Chronic Pathologies Following Ocular Sulfur Mustard Exposure: A Novel Injury Modality and Therapeutic Target

P. McNutt and M. Lyman. *USAMRICD, Gunpowder, MD.*

Following ocular exposure to moderate or high doses of the chemical warfare agent sulfur mustard (SM), many victims develop a persistent disease known as mustard gas keratopathy (MGK), involving a keratitis of unknown etiopathogenesis with secondary keratopathies such as recurring epithelial lesions, corneal neovascularization and progressive corneal degeneration. The pathophysiological origin of MGK is unknown, and diverse therapeutic approaches have completely failed to prevent MGK onset, suggesting that researchers need an improved understanding of the mechanisms underlying injury progression. While the effects of SM on the corneal epithelium are well understood, the effects on corneal endothelium are completely unknown. The corneal endothelium is a monolayer of cells that maintain the cornea in a dehydrated state through acting as a semi-permeable barrier and osmotic pump. Disrupted endothelial function results in clinically untreatable clinical diseases. Hypothesizing that endothelial toxicity may present a novel injury modality that could explain the idiotypic etiogenesis of MGK, we characterized endothelial integrity and function using a well-described rabbit model of corneal vapor exposure that exhibits acute and long-term sequelae commensurate with human outcomes. Functional, ultrastructural, immunological and cytochemical methods converged to demonstrate that ocular SM vapor exposure results in acute endothelial toxicity, gross disruption of endothelial barrier function and long-term endothelial pathologies in MGK eyes. Furthermore, we demonstrate that the severity of endothelial toxicity is determinative of MGK onset and progression. These data indicate that endothelial toxicity occurs at the right time and with the appropriate pathophysiology to contribute to MGK and furthermore explicate many of the previously confusing aspects of clinical progression in SM-injured eyes. Based on these findings, we propose a new model of corneal injury that synthesizes what is known regarding the relationship between SM dose, the biphasic clinical progression and the various clinical trajectories of corneal SM injury to explicate the etiology of MGK onset.

1603 Ocular Irritation Risk Assessments Using *In Vitro* BCOP (OECD 437) and 3D Assays (OECD 492)

C. S. Roper, J. L. Vinall and J. B. Welch. *In Vitro Sciences, Charles River Laboratories, Tranent, United Kingdom*. Sponsor: S. Bulera.

Ocular safety testing classifies compounds according to the United Nations Global Harmonized System of Classification and Labeling of Chemicals (UN GHS) as No Category (non irritant), Category 2 (irritant) or Category 1 (corrosive/ irreversible damage). A number of alternatives to the rabbit eye irritation test (OECD 405; Draize) are now in use, based on the principles of reduction, refinement and replacement. The most frequently used models are the BCOP assay (OECD 437) and the 3D reconstructed tissue models; MatTek EpiOcular and SkinEthic HCE (OECD 492). Each of the *in vitro* assays predicts some of the UN GHS categories, however, none of the available validated non-animal alternatives are universally applicable. BCOP assigns classifications of No Category, No prediction can be made and Category 1. The 3D models assign classifications of No Category and Category 1/2. Together, these *in vitro* models are being used in top-down and bottom-up approaches for risk assessment. The OECD test guidelines require that testing laboratories demonstrate technical competence with a proficiency panel of solid and liquid test chemicals. For BCOP, the proficiency panel contained 5 No Category, 3 No prediction can be made and 4 Category 1 chemicals. All chemicals were correctly assigned based on the resultant *in vitro* irritation score (IVIS). For EpiOcular, the proficiency panel contained 7 No Category and 8 Category 1/2 chemicals. All chemicals were correctly assigned based on viability measured by an MTT assay. For HCE, Charles River participated in a multisite ECVAM style validation. A total of 60 chemicals (30 solid and 30 liquid) were blind tested on 3 occasions each by the participating laboratories. The chemicals covered all GHS classifications. The defined acceptance criteria were achieved for both solids and liquids. Charles River have demonstrated technical proficiency in BCOP, EpiOcular and HCE assays. In-house fit-for-purpose validation data confirmed that BCOP and EpiOcular assays correctly assigned ocular irritation potential of the proficiency panels of chemicals. While neither method alone is capable of fully classifying substances in UN GHS categories, a strategic combination of these tests in a tiered approach can be used to generate UN GHS ocular irritation categorization.

1604 Histopathological Findings on the Cornea in the Bovine Corneal Opacity and Permeability Test (BCOP Test) for Alternative to Eye Irritation Test

M. Furukawa[1], T. Sakakibara[1], K. Ito[1], S. Sasaki[1], M. Koshita[1], S. Okumura[1], K. Kawamura[1], M. Matsuura[1] and H. Kojima[2]. [1]*Safety Research Institute for Chemical Compounds, Co., Ltd, Sapporo, Japan and* [2]*Japanese Center for the Validation of Alternative Methods, National Institute of Health Sciences, Tokyo, Japan.*

Background: The Bovine Corneal Opacity and Permeability (BCOP) test (OECD TG437) in an isolated bovine cornea is an alternative method of eye irritation test that can be used for evaluation of ocular non-irritants (Non GHS Category) and corrosives and severe irritants (GHS category 1). In alternative eye irritation tests including BCOP test, there is no test system capable of evaluating GHS Categories 2A and 2B including mild or moderate irritants. We have been considering the possibility on the evaluation of mild or moderate irritants in the BCOP test with a combination of histopathological examination, and have presented development of histopathological findings in the corneal epithelium and the stroma caused by the test substance. Consequently, we tested approximately 60 substances with Non GHS Categories, and GHS 2A and 2B to investigate the possibility on the evaluation of mild or moderate irritants based on the results of IVIS (*in vitro* irritation score), which is the evaluation criteria of BCOP test, and pathological findings in the cornea (corneal epithelium and stroma). Methods: The IVIS values were calculated based on the changes in the corneal opacity and permeability values following exposure to the test substance according to the ordinary method. After the opacity and permeability determinations, corneas were fixed in formalin and stained with HE and pathologically examined. Results: Histopathological examination of the cornea revealed the following findings: (1) IVIS less than 3: No changes or changes only at the epithelial surface. (2) IVIS around 10: Changes in the epithelial basal cell layer. (3) IVIS 25 to 40 and 40<: Changes in all layers of corneal epithelium and stroma. There results suggest the possibility that a substance causing pathological findings localized at the corneal epithelia is a mild or moderate irritant.

1605 An Adaptive Study Design to Evaluate for the Onset, Progression, and Reversibility of Lenticular Changes in Wistar Han Igs Rats

R. Yeager[2], J. Johnson[2], J. Wetter[2], Y. Lao[2], J. Ford[1], J. Ruger[1], J. Bartoe[1] and T. Hudzik[2]. *[1]MPI Research, Mattawan, MI and [2]Drug Metabolism, AbbVie, Inc., North Chicago, IL; [3]Preclinical Safety, AbbVie, Inc., North Chicago, IL.*

During a three-month general toxicology study in Wistar Han IGS rats, test article (TA)-related lenticular changes were identified during clinical observation and confirmed as cataracts by slit-lamp biomicroscopy. The lenticular changes were unexpected, given the target biology/pharmacology and lack of ocular findings in previous six- and nine-month studies in Wistar Han rats and Beagle dogs, respectively. To evaluate the onset, progression, and potential reversibility of the lenticular change, rats were orally administered vehicle or TA (n=20/sex/group) and once weekly ophthalmoscopic examinations were conducted by a board certified veterinary ophthalmologist. Based on ophthalmoscopic findings, animals were assigned to one of three phases (onset, progression, or reversibility) with an equal proportion of animals in each phase. Onset of lenticular changes occurred by Week 4 of dosing. Progression of lenticular changes included 1) swelling/splitting of the posterior cortical suture lines; 2) radially oriented anterior and posterior lenticular opacities; 3) triangular-shaped opacification of the posterior cortex in the region of the suture lines; 4) opacification of anterior and posterior cortical fibers to the point of obscuring fundus examination; and/or 5) prominence of the lenticular nuclear/cortical junction. Histopathology findings consisted of minimal to moderate swelling or swelling/degeneration of lens fibers. A majority of recovery phase animals developed a prominence of the lenticular nuclear/cortical junction, with mild to moderate lens fiber degeneration. In conclusion, assigning animals to study phase (onset, progression, or recovery) based on weekly ophthalmoscopic findings afforded an adaptive study design to characterize the time course of TA-related lenticular changes in rats.

1606 Optical Coherence Tomography (OCT) Correlating with Histopathology for Peripheral Retina Degeneration in a Chronic Monkey Toxicity Study

L. Mueller, B. Jacobsen, A. Herrmann and B. Altmann. *F. Hoffmann-La Roche, Basel, Switzerland.*

Optical coherence tomography (OCT) has been used within toxicity studies to evaluate clinically findings of the retina, and the findings have been compared with the corresponding histopathological findings in the eye. Usually this is performed in studies where the compound is administered intra vitreal. However, usually the central region of the eye is evaluated only. Here we describe retinal findings within a toxicity study of an oral administered compound in monkeys present at the periphery and it's matching OCT findings. During a chronic toxicity study in monkeys of a heterocyclic compound derived from a substituted coumarin chemotype, retinal degeneration was observed after 39 weeks of daily oral dosing. The lesion was seen in terminal sacrificed main study animals histologically but not during routine ophthalmoscopic examinations. Histopathologically, the most affected layers were the outer nuclear layer and photoreceptor layer. Incidence and severity were dose dependent and mostly located in the periphery. OCT was applied to the recovery animals of the study at 13, 20 and 26 weeks of recovery and revealed abrupt destruction of the photoreceptor layer and blurring of the outer nuclear layer in 3 out of 4 animals with comparable results for the individual animals across the three time points. Histopathological examination performed at the end of the recovery period revealed retinal degeneration correlating very well with the OCT results regarding incidence, location and severity. In particular, it was noteworthy that the one recovery animal, which did not have abnormal findings in OCT, did not show any evidence for retinal degeneration as well. Thus, OCT is a suitable imaging mode to detect and monitor retinal changes even in the far periphery of the retina. However, OCT conditions had to be adapted to assess the periphery of the retina where the lesions occurred.

1607 Application of Optical Coherence Tomography (OCT) for Examination of the Posterior Segment of the Eye in the Beagle Dog

E. Grosdidier[1], J. Silvano[1], A.-S. Augsburger[2] and R. Forster[1]. *[1]CITOXLAB, Evreux, France and [2]DMV Clinique Vétérinaire, Bois Guillaume, France.*

Intravitreal and subretinal administrations are associated with a higher incidence of changes in the posterior segment of the eye in all species including Beagle dogs. Limited non-invasive technologies are available in preclinical toxicology and optical coherence tomography (OCT) has become a leading approach to acquire high-resolution images of the retina and optic nerve enabling a structural evaluation of the posterior ocular segment. While cooperation of patients facilitates imaging in humans, OCT imaging in animals presents species specific challenges that require adaptation in laboratory animals as this ocular evaluation is performed under anesthesia. A review of critical components in standardization of acquisition protocol was undertaken. Injectable short anesthesia, body and ocular positioning methods/tools and ocular preparation were identified as key success factors in the Beagle dogs. Immobilization of the ocular globe with positioning of the papilla in the acquisition axis of the imaging equipment was essential along with corneal rehydration. Images were obtained with x6 magnification, grid pattern and medium to low light intensity. The application of these practical considerations enabled identification of retinal layers and reference measurements of the retinal thickness in Beagle dogs are presented with the imaging conditions selected. Average values for retinal thickness of adult healthy Beagle dogs were 190 microns with a standard deviation of 14 microns. Minimal variations were observed in baseline measurements between both eyes from the same animal.

1608 Coefficient of Variation in the Electroretinographic Response of Laboratory Animals

J. N. Ver Hoeve[2,4], C. B. Kim[2,4], T. M. Nork[2,4], J. S. Eaton[2,5], B. Christian[1] and C. J. Murphy[2,3,5]. *[1]Covance Laboratories, Inc., Madison, WI; [2]Ocular Services On Demand (OSOD), Madison, WI; [3]Dept. of Ophthalmology & Vision Sciences, University of California-Davis, School of Medicine, Sacramento, CA; [4]Dept. of Ophthalmology & Visual Sciences, University of Wisconsin-Madison, School of Medicine & Public Health, Madison, WI and [5]Dept. of Surgical & Radiological Sciences, University of California-Davis, School of Veterinary Medicine, Davis, CA.*

Purpose: To compare variability in the amplitudes of the dark-adapted electroretinogram (ERG) among commonly used species of laboratory animals. Methods: All ERGs were collected from normal eyes, naive to any treatment or surgical intervention. Species tested included monkeys (cynomolgus, N=160), dogs (beagle, N=142), minipigs (Yucatan, N=40), pigmented rabbits (Dutch belted, N=349), albino rabbits (New Zealand white, N=411), albino rats (Sprague Dawley, N=84), and mice (C57/BL6, N=32). All animals were dark-adapted for at least 2 h, anesthetized, and had their pupils dilated prior to testing. ERGs were recorded in response to 2.5 cd-s m^-2 flashes, delivered in a large ganzfeld dome. ERGs were digitally filtered, and B-wave amplitudes computed automatically. B-wave amplitudes from one eye of each animal were entered into the analyses. Results: Coefficients of variation (standard deviation/mean, COV) for B-wave amplitude ranged from 0.21-0.37 across species. COV was lowest for the cynomolgus monkey (0.208), followed by the beagle dog (0.250). COV was highest for the albino rat and C57/BL6 mouse (both 0.370), followed by the minipig (0.350) and pigmented rabbit (0.340). The shape of the B-wave amplitude distributions was examined under various transforms. The distribution of B-wave amplitudes approximated a normal distribution. A log transform or square-root transformation of the data may improve the fit to a normal distribution. Conclusions: Nonclinical safety and efficacy studies of ocular drugs and devices frequently use ERG to non-invasively assess the functional status of the retina. Using the COV, we show that the variability in various commonly used laboratory species is lowest for monkeys and dogs and slightly higher for rabbits and rodents. These data can be used to sufficiently power studies, inform study design, and facilitate interpretation and comparison of ERG results both within and between laboratory species.

1609 Reduced Disc Shedding Contribute to Kava Kava Extract-Induced Retinal Degeneration in F344/N Rats

H. Yamashita[3,5], M. J. Hoenerhoff[3], K. R. Shockley[1], S. D. Peddada[1], K. E. Gerrish[2], D. Sutton[3], C. A. Cummings[4], Y. Wang[3], J. F. Foley[3], M. Behl[3], R. C. Sills[3] and A. R. Pandiri[3]. *[1]Biostatistics and Computational Biology Branch, NIEHS, Research Triangle Park, NC; [2]Molecular Genomics Core Laboratory, NIEHS, Research Triangle Park, NC; [3]National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC; [4]UltraPath Imaging, Research Triangle Park, NC and [5]Drug Safety, Taisho Pharmaceutical Co., Ltd., Saitama, Japan.*

Kava kava extract (KKE) derived from the root of the tropical shrub Piper methysticum was originally used for ceremonial beverages in the South Pacific and is currently used as an herbal alternative to anti-anxiety drugs. In the 2-year National Toxicologic Program (NTP) KKE gavage bioassay in F344/N rats, the incidence of retinal degeneration was significantly increased in a treatment-related fashion in both sexes. A retrospective evaluation of rat retinas from the 2-year NTP KKE bioassay indicated the distribution and morphology of retinal degeneration was similar to the changes observed in light-induced retinal degeneration reported in albino rats. Therefore, it was hypothesized that KKE has a potential to exacerbate the light-induced retinal degeneration. To investigate the mechanism of retinal degeneration, we conducted a 90-day F344/N rat KKE gavage study at doses of 0 and 1.0 g/kg (highest dose in 2-year NTP rat KKE bioassay). The aim of the this study was to 1) examine the effect of KKE exposure on pupil dilation, 2) evaluate the morphological alterations in the retina by light and electron microscopy, and 3) evaluate the transcriptomic alterations in the retina by microarray. The morphological evaluation indicated a decrease in disc shedding and subsequent reduced phagocytosis of rod outer segments in the retinal pigmented epithelium of the superior retina. Transcriptomic alterations related to melatonin and serotonin signaling were observed on microarray analysis. Disc shedding is essential to maintain the homeostasis of the photoreceptor layer and is regulated by dopamine, melatonin and serotonin signaling. Therefore, it is thought that reduced disc shedding and alterations in the melatonin and serotonin pathways contribute to the increased incidences of retinal degeneration in KKE exposed F344/N rats.

1610 Attributes of the Tear Film in Species Used in Preclinical Ocular Toxicology Investigations of Dry Eye Disease (DED)

A. D. Rodrigues[3], J. S. Eaton[1,2] and C. J. Murphy[1,2]. *[1]Ocular Services On Demand, Madison, WI; [2]Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California Davis, Davis, CA and [3]Non-Clinical and Translational Sciences, Allergan, Irvine, CA.*

The precorneal tear film (PTF) is dynamic, has numerous constituents, contributes to the maintenance of ocular surface health, and is a barrier to infection. Insufficiency or imbalance can result in tear film instability and dry eye disease (DED). Animal models for toxicity testing are best chosen with understanding of the components of the PTF. Here, we review the published attributes of the tear film of the rabbit and dog and draw comparisons to reported values for humans. References were identified using online publication databases including PubMed, Medline and Google Scholar as well as books and journals accessible through UC Davis Health Sciences Libraries. Over 100 reports were identified spanning from as early as 1954 to present-day. Normative values for the following parameters were identified: total tear film thickness, lipid layer thickness, tear volume, tear flow rate, tear break up time (TBUT), and Schirmer tear test I (STT I). Lipid layer & total tear film thickness are greater in rabbits (0.18µm & 10µm) than in humans (0.08µm & 4µm). For dogs, lipid layer thickness was reported but data was limited (0.1µm); total tear film thickness was not reported. Tear volume in rabbits is similar to that of humans (7-8µL), but data was not found in dogs. Reported values for TBUT in rabbits varied (20-55sec), with similar values reported for dogs (19sec) and humans (18sec). Values for tear production as reflected by STT I results were lower in rabbits (8mm/min), and greater in dogs (21mm/min) than humans (19mm/ 5min). Tear flow rate varied between rabbits (2.5µL/min), dogs (11µL/min), and humans (1µL/min). Of the major differences between species, large thickness of lipid layer in rabbits, and high tear flow rate in dogs were the most dramatic. Normative values are important for choosing a relevant species for preclinical toxicology studies, and to enhance translatability of results. A knowledge gap exists regarding fundamental physiochemical attributes of the tear film of species commonly used in development of palliatives and therapeutics for DED.

1611 *In Vitro* Assessment of the Inflammatory Potential of Ocular Delivery Systems

A. Cercillieux, F. Lorget, C. Farman, S. Laing, J. A. Aaronson, A. Famili, D. Chang, K. Rajagopal, E. Thackaberry and K. Staflin. *Pharma Technical Development Drug Delivery, Genentech, South San Francisco, CA; Safety Assessment, Genentech, South San Francisco, CA.*

The treatment for diseases such as age-related macular degeneration (AMD) poses a challenge in that monthly or bi-monthly intravitreal (IVT) injections are often necessary to sustain drug exposure. To mitigate this, biocompatible/biodegradable polymer based materials (e.g. Poly(lactic-co-glycolic acid) (PLGA)) are being considered for long acting delivery systems (LAD). However, these materials have the potential to trigger inflammation and induce foreign body reaction (FBR) in various tissues including the eye. Here, we developed *in vitro* tools to investigate inflammatory responses to biomaterials and identified soluble mediators of FBR. Fourteen cytokines/chemokines were examined after treating human peripheral blood mononuclear cell (PBMC) cultures with PLGA microspheres and other biomaterials with different surface chemistry. Temporal and quantal differences in cytokine/chemokine responses were detected with up-regulation of IL-1b, IL-8, TNFa, MCP-1, MIP-1a, MIP-1b and RANTES. Furthermore, the soluble players identified by *in vitro* assessment were similarly up-regulated in the rabbit eye administered with the same biomaterials as assessed by *in situ* hybridization. The data suggests a predominant innate immune response associated with FBR in the eye. In addition, an *in vitro* assay that captures the formation of multi-nucleated giant cells by macrophage fusion was established where differential responses to biomaterials were observed including phenotypic changes, cytokine and chemokine production as well as transcriptional changes. Overall, the *in vitro* results suggest *in vivo* translatability and potential immune players associated with FBR in the eye. Ultimately, these *in vitro* assays may be useful as potential tools to investigate mechanism of toxicity for long-acting ophthalmologic drug delivery systems and help improve current chemistry to minimize these responses.

1612 Assessment of the Predictive Capacity of SkinEthic HCE Human Corneal Test Method for Serious Eye Damage/Eye Irritation Assessment: A Depth Analysis Based on the Drivers of UN GHS Classification

N. Alepee, V. Leblanc, M. H. Grandidier, L. Nardelli, G. Swaka and J. Cotovio. *L'Oreal, Aulnay Sous Bois, France.* Sponsor: E. Dufour.

Recent papers have shown the importance of thorough understanding of serious eye damage/eye irritation effects for the evaluation of alternative methods relevance. Based on a full description of all the ocular effects that drive classification available for a large set of reference chemicals (Adriaens et al., 2014; Barroso et al., 2015) a concordance analysis with *in vitro* prediction results obtained with the SkinEthic™ HCE, a reconstructed human corneal test method is of major interest. In order to evaluate the predictive capacity of the SkinEthic™ HCE test method and its limitations, a set of 191 substances (103 UN GHS No Category, 42 Category 2, and 46 Category 1) were correlated with the *in vivo* drivers of classification. Results show that 96.1% of *in vivo* UN GHS Cat. 1 chemicals (25 liquids and 21 solids) that were classified with the SkinEthic™ HCE method had corneal opacity (CO) mean ≥ 3 (mean scores at day 1, 2 and 3 after instillation of the chemical in the eye) and CO persistence on day 21 (in the absence of severity as main drivers classification. Overall, 84.8% of the *in vivo* UN GHS Cat. 2 chemicals (25 liquids and 17 solids) were always correctly predicted dividing up into the 2 described main drivers classification: CO mean ≥ 1 and conjunctival redness mean ≥ 2. Among the 103 *in vivo* UN GHS not classified chemicals, 76% were correctly predicted with main driver classification CO=0. Interestingly liquid chemicals were often over-predicted of the subgroups CO>0 where this relationship was not observed for the solids (>85% correctly predicted). In conclusion, the analysis of 191 chemicals, among which 120 substances tested in the SkinEthic™ HCE multicentric studies, shows a proportioned repartition of the most important drivers of Category 1, Category 2 and No Category classification consolidating the relevance of the test method.

1613 Use of HPLC/UPLC-Spectrophotometry for Detection of Formazan in SkinEthic HCE Reconstructed Human Corneal Test Method to Expand Its Applicability to Strongly Coloured Chemicals

M. H. Grandidier, L. Nardelli, M. Talleur, J. Cotovio, V. Leblanc and N. Alepee. *L'Oreal, Aulnay Sous Bois, France*. Sponsor: E. Dufour.

Assessment of the potential of a chemical to produce serious eye damage/eye irritation in Reconstructed human Tissues test methods is based on tissue viability that is determined by enzymatic reduction of MTT to formazan. Up to now, formazan is quantified photometrically with the results expressed as % viability of the chemical relative to the negative control. A known limitation of the photometric MTT-reduction assay is possible interference of coloured chemicals with the absorbance measurement of formazan (SCCS, 2010; Alépée et al, 2015). The purpose of the current study was to demonstrate the concordance of using an analytical method - HPLC/UPLC-spectrophotometry - for endpoint detection in the SkinEthic HCE test method on liquid and solid chemicals to expand it applicability to coloured/strong MTT reducers chemicals. The viability of 24 chemicals was quantified by photometric and HPLC/UPLC-spectophotometry. Among the 11 liquid and 13 solid chemicals, pre-check showed that 4 test substances were MTT reducers and 11 were MTT reducing colour interfering chemicals. Photometric measurements could not be achieved for 5 out of 24 chemicals due to an incompatibility (overflow results obtained using optical density endpoint parameter) or a difference > 20 for the adapted controls using optical density endpoint parameter. The results of the remaining 19 chemicals demonstrate that very similar or identical viability measurements were obtained for both approaches since the viabilities were close to the line of unity (y=0.98x+0.88). In addition, the regression line coincides with the line of unity, with a coefficient of determination (R2) value of 0.99. This result supports the findings of the study that HPLC/UPLC-spectrophotometry can be applied for SkinEthic HCE test method, actually expanding its assessment of serious eye damage/eye irritation effects to any strong MTT/colour chemicals.

1614 Intra- and Inter-Laboratory Evaluation of SkinEthic™ HCE Test Method on Solid and Liquid Chemicals for Serious Eye Damage/Eye Irritation Assessment

V. Leblanc[3], E. Adriaens[1], M. H. Grandidier[3], K. Hollanders[4], A. Jacobs[4], M. Meloni[5], L. Nardelli[3], C. Roper[2], E. Santirocco[5], F. Toner[2], A. Van Rompay[4], J. Vinall[2], J. Cotovio[3] and N. Alepee[3]. *[1]Adriaens Consulting, Aalter, Belgium; [2]Charles River Laboratories, Edinburgh, United Kingdom; [3]L'Oreal, Aulnay Sous Bois, France; [4]Vito NV, Mol, Belgium and [5]Vitroscreen, Milano, Italy*. Sponsor: E. Dufour.

Human reconstructed tissues methods have been suggested for being part of tiered strategies for *in vivo* Draize test replacement allowing identification of severe eye damage (category 1)/eye irritation (category 2) and no classified (No Category) chemicals according to UN GHS. SkinEthicHCE test method, based on Human corneal epithelium cells, has been developped to discriminate liquid and solid chemicals not requiring classification for serious eye damage/eye irritancy from those requiring it. Two multicentre studies (for liquids and solids) were conducted in three laboratories to evaluate its (1) reliability: within (WLR) and between (BLR) laboratories reproducibility and (2) relevance. Protocols consist of topical exposure of chemical onto the SkinEthic HCE test system followed by adapted time incubation and cell viability measurement. Briefly, 2 distincts sets of 60 chemicals each were tested in double-blind studies. The average WLR from the 3 laboratories was 92.2% for liquids and 95.6% for solids while BLR was 93.3% irrespective to the protocols. Based on bootstrap resampling, the specificity (No Cat) of the SkinEthic HCE test method was 73.8% for liquids and 76.6% for solids. The sensitivity (Cat. 1/Cat. 2) for liquids and solids was 98.2% and 91.9% with an accuracy of 86.8% and 84.3% respectively. At present, 200 chemicals were tested (105 liquids and 95 solids) resulting in a sensitivity of 95.2%, specificity of 72.1% and accuracy of 83.7%, thereby meeting all acceptance criteria for predictive capacity. In conclusion, the SkinEthic HCE test method is an efficient transferable and reproducible assay, integrable into a battery of assays for eye irritation risk assessment.

1615 Using the Short Time Exposure (STE) and Eye Irritation Test (EIT) for Full GHS Categorization of Ocular Irritation

J. A. Willoughby[2], B. Meyer[2], L. Blakeman[2], B. Franz-Jonas[2] and C. Dilworth[1]. *[1]Cyprotex PLC, Macclesfield, United Kingdom and [2]Cyprotex US, LLC, Kalamazoo, MI*. Sponsor: C. Strock.

The United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS) has defined 3 primary categories for ocular irritation. Ocular corrosives are Category 1, ocular irritants are Category 2 and non-irritants are defined as No Category. Though there are a number of OECD validated *in vitro* ocular irritation assays available, none have been capable of fully categorizing chemicals or compounds. In fact, these *in vitro* assays predict either Category 1, No Category, or a combination of Category1/Category 2, with no separation between the two categories. The goal of our study was to determine if the *in vitro* Short Time Exposure (STE; OECD 491) assay, which categorizes Materials as No Category, Unable to Classify, or Category 1, in combination with the MatTek Eye Irritation Test (EIT; OECD 492) which categorizes Materials as No Category or a combination of Category 1/Category 2, could be used together in a tiered approach to fully categorize potential ocular irritants. To that end, the 15 proficiency compounds specified in OECD 492 were assessed in both the STE and EIT assays. The proficiency compounds consist of 4 *in vivo* Category 1 compounds, 4 *in vivo* Category 2 compounds and 7 *in vivo* No Category compounds. The 15 compounds were first assessed in the EIT assay. All 15 of the proficiency compounds were correctly categorized into the two possible categories (No Category or Category1/Category2). These 15 test materials were then assessed in the STE assay. When the data from the two assays was combined it showed that most of the 15 proficiency compounds were properly categorized according to the known *in vivo* UN GHS categories. Though accurate, numerous test materials still should be assessed in both of these assays simultaneously to determine potential limitations of combining the two assays. Finally, these data suggest the combination of the EIT and STE methods, in a tiered approach, can accurately categorize ocular irritants into all three UN GHS ocular irritation categories.

1616 Critical Review of the UN GHS Decision Criteria for Chemicals Causing Irreversible Effects on the Eye/Serious Damage to the Eye: Cosmetics Europe Analysis

M. Klaric[4], U. Pfannenbecker[2], E. Adriaens[1], N. Alépée[7], J. Barroso[5], J. Hibatallah[3], K. R. Mewes[6] and P. McNamee[8]. *[1]Adriaens Consulting, Aalter, Belgium; [2]Beiersdorf AG, Hamburg, Germany; [3]Chanel Parfums Beauté, Neuilly sur Seine, France; [4]Cosmetics Europe, Brussels, Belgium; [5]European Commission Joint Research Centre, Ispra, Italy; [6]Henkel AG & Co. KGaA, Düsseldorf, Germany; [7]L'Oréal Research & Innovation, Aulnay, France and [8]The Procter & Gamble Company, Egham, United Kingdom*. Sponsor: A. Schepky.

A thorough understanding of which of the effects assessed in the *in vivo* Draize eye test are responsible for driving UN GHS classification is critical for an adequate selection of chemicals to be used in the development and/or evaluation of *in vitro* methods and for properly assessing test method predictive capacity and limitations. For this reason, Cosmetics Europe has compiled a database of Draize data (Draize eye test Reference Database, DRD) from external lists that were created to support past validation activities. The DRD contains 165 independent Category 1 (Cat 1) studies. An in depth analysis of the Cat 1 studies classified based on persistence of effects in the minority/majority of animals (77 studies) or on corneal opacity (CO) = 4 (34 studies) was performed. UN GHS classification criteria define that a Cat 1 classification can be triggered based on tissue effects observed in a single animal. It is questionable whether such results should lead to a Cat 1 classification, especially in case of delayed effects observed in a single animal in the absence of any other Cat 1-triggering effects. Furthermore, analysis of chemicals that were tested in more than one study (for Cat 1: 16 chemicals tested in 37 studies) confirmed in many cases that chemicals classified as Cat 1 by persistence or CO = 4 in a single animal were classified by a different driver in the repeat studies. The evidence suggests that a critical revision of the UN GHS Cat 1 decision criteria should take into account that persistence of effects and CO = 4 should be present in >60% of the animals to drive a classification.

1617 28-Day Repeated Dose Intravenous Toxicity Study of Combination Excipient [5% Pharmasolve™ + 45% Propylene Glycol + 50% Polyethylene Glycol 400] in Wistar Rats

V. Goyal, S. Pandey, S. Kakade, P. Are, P. Nalge, V. Sthevaan, U. Bijegaonkar and R. Nirogi. *Discovery Toxicology, Suven Life Sciences Ltd, Hyderabad, India.*

Safety evaluation of chemicals in animals is one of the key components of drug development. In the early phases of preclinical drug development, new chemical entities are seldom entirely optimized and may be difficult to solubilize. Consequently, the use of various excipients is required to formulate the test compounds for *in vivo* administration. Drug should be administered in animals by the same route as intended for humans. Some of the drugs are administered via extravascular routes because of compound nature and IV administration is also a prerequisite to characterize pharmacokinetics of a drug. Vehicle used for IV administration must have some definitive properties. Many solvents are being used as standalone for IV solutions but combination of 2 or more solvents are also not uncommon. There is paucity of data on the safety of combination excipients therefore the present study was designed with the objective to evaluate the effects of combination vehicle used for intravenous administration n rats when given for consecutive 28 days. In this study, combination of three commonly used co-solvents viz., Pharmasolve, Propylene glycol and Polyethylene glycol PEG 400 were tested at the ratio of 5%, 45% and 50% respectively, for adverse clinical reactions and effects on hematology, clinical chemistry parameters, gross and histopathology. Combination excipient was administered intravenously via lateral tail vein to four different groups (0.5, 1, 2 and 5 mL/kg) comprised of 5 rats per sex. Control rats were administered with physiological saline solution. At 5 mL/kg, convulsion, abnormal locomotion and death was noticed in few rats. No significant changes on body weight, feed consumption, hematology or serum clinical chemistry and gross or histopathological examination were noted up to 2 mL/kg. It is therefore concluded that this combination up to 2 mL/kg dose volume can be used for 4-week rat studies.

1618 Emerging Biologics Development Methods: Free-Ranging Cynomolgus Monkeys Undergoing Continuous Intravenous Infusion with an Implanted Port Catheter System–A Method Transfer in Courtesy to the "Refinement" in the 3 R's

C. Miller[1], S. Korte[2], A. Salva[1], N. Snippe[2], F. Runge[2] and H. van Wijk[1]. [1]*Covance Laboratories Harrogate, Harrogate, United Kingdom and* [2]*Covance Preclinical Service GmbH, Münster, Germany.*

Numerous pharmaceuticals and recently an increasing list of monoclonal antibodies undergoing regulatory pre-clinical trials in non-human primates are administered intravenously, often by continuous infusion. By courtesy to the "Refinement" in the 3 R`s, an existing infusion technique was required to be transferred to a new laboratory setting. During a pre-clinical safety assessment study, one control monkey of each sex (*Macaca fasicularis*, 2.9 - 3.9 kg, Mauritius) underwent port catheter surgery. The subcutaneous port (PortHold and accompanying needles, Access Technologies, USA) was implanted with the tip being inserted in the Vena cava caudalis. Dosing (Model 500 V Pump (Pegasus®), Orchesta Infusion, USA) commenced following a 4 week recovery, system flushing with NaCl (incl. a heparin block [10 IU/mL], 0.69 mL dead space), jacket training, pump precision test and pre-study diagnostics. The animals were free-ranging (max. 20% back pack vs body weight ratio) administered continuously for 24 hours and were recorded with stable body weights throughout the training and dosing period. Housing and dosing was conducted under ETS1231) conditions and clinical signs during the recovery period were limited to minimal swollen inguinal areas. Post-surgery week 4 clinical pathology proved the absence of inflammatory processes before start of dosing. Port patency was given for the complete duration of the study and great precaution was implemented to conduct port-needle changes under aseptic conditions. The successfully transferred infusion technique, now allows the conduct of further dose regimens in the pre-dominant non-human primate species for the preclinical safety assessment of biologics. 1) ETS 123, 2007: European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Appendix A. Official Journal of the European Communities K, 2525.

1619 Site-Specific Covalent Chemical Ligation Strategy for Monoclonal and Polyclonal Immunoglobulins at the Nucleotide Binding Sites

D. Lac[2], S. C. Feng[2], G. Bhardwaj[2], H. Le[1], J. Tran[1], L. Xing[1], G. Fung[2], R. Liu[2], H. Cheng[1] and K. S. Lam[2]. [1]*University of California, Davis, Davis, CA and* [2]*University of California, Davis, School of Medicine, Sacramento, CA.* Sponsor: K. S. Lam.

Background: Targeted therapy using monoclonal antibodies (mAbs) has revolutionized the treatment of cancer by recognizing antigens expressed on cell surfaces. Creating antibody-drug conjugates (ADC) combines the targeted specificity of mAbs with the enhanced tumor-killing power of toxic effector molecules results in fewer toxic side effects than most conventional chemotherapeutic drugs. Drugs are conjugated to antibodies through nonspecific methods, via primary amines from lysine or free sulfhydryls from cysteines. However the heterogeneous product generates variable pharmacokinetics, efficacy and safety profile. Therefore, to create a homogenous product, extensive purification processes are typically needed. Current site-specific conjugation methods include both antibody engineering and chemical methods. Here we have exploited the use of a ligand that binds directly to the Fab arms of the immunoglobulin molecule. Methods: One-bead-one-compound (OBOC) combinatorial library was designed based on the known molecule, which has a strong binding affinity with nucleotide binding pocket presented in the Fab arm of all the immunoglobulin. The computational docking gave *in silico* screening at the same time. The lead hit has the top score from both screening will be resynthesized in the solution phase for further characterization. Results: 1,5-difluoro-2,4-dinitrobenzene with additional two amino acids presents the optimal binding ability. Average two crosslinkers attaches to the surface per antibody. Fragmentation experiment indicated the conjugation only happened at the Fab arm. The antibodies also retain all the biological mechanism after conjugation including antigen-binding affinity, molecular mechanism and internalization ability. The ADC generated by this technique demonstrates target specific cellular toxicity in several different receptor expressed cancer cell lines. Conclusion: Compared to other site-specific covalent modifications, this technique is mild, highly efficient, and can be used for many clinically available monoclonal, polyclonal antibodies. Applying this technology to generate immunoconjugates would lead to less toxicity and higher antibody instability with more homogeneous pharmacokinetics and safety profile.

1620 Safety Evaluation of 2-Deoxy-D-Glucose (2DG) Following 28 Day Oral Gavage Administration in Beagle Dogs

K. E. Elsass. *Life Sciences Research, Battelle, West Jefferson, OH.*

2DG is a potential anticonvulsant and disease-modifying agent for epilepsy patients. A study was conducted to assess the safety of 2DG, gavaged either with vehicle or 2DG (5, 30, or 90 mg/kg BID; total dose of 10, 60 or 180 mg/kg/day) for 28-consecutive days with a 14-day recovery period. Endpoints evaluated included clinical observations, body weights, body temperatures, food consumption, ophthalmic examinations, ECG and respiratory measurements, clinical pathology, cardiac biomarker NT-pro-BNP, and histopathology. Plasma 2DG was measured for PK evaluation. Necropsies were performed on days 29 and 43. Abnormal clinical observations of yellow feces, soft feces and/or diarrhea were observed. Although these changes were intermittent, the incidence was increased with an increase in dose affecting 2/10, 3/10, 6/10, and 9/10 total dogs in the vehicle, 10, 60, and 180 mg/kg/day dose groups, respectively. For day 1, no apparent sex-dependent differences in systemic exposure of 2DG were noted as Cmax and AUC values were within 50 percent between males and females and these values increased in an approximately dose-dependent manner. There was no increase in systemic exposure noted on day 28 when compared to day 1. Increases in aspartate aminotransferase (AST) values (107 to 166 percent) were noted on days 15 and 29 in high dose males and females. Histopathology analysis showed cytoplasmic alteration in the liver of one male and one female dog administered 180 mg/kg/day 2DG on day 29. On day 43, cytoplasmic alteration was observed in the liver of one high dose male indicating trend towards recovery. AST changes and the liver microscopic finding were considered non adverse due to small magnitude with minimal severity; as such, the no-observed-adverse-effect level was considered to be 180 mg/kg/day with a mean AUC of 188 hr*µg/mL (Tmax 44 minutes). (Supported by LEIDOS Contract No. HHSN261200800001E, and NINDS under BrIDGS Program).

1621 Hyperglycemia Potentiates Antiretroviral Drug-Induced Mitochondrial Toxicity in Blood-Brain Barrier Endothelium: A Critical Need for Safety Evaluation of HAART Therapy in Diabetics?

R. K. Sajja, S. Prasad, M. A. Kaisar and L. Cucullo. *Pharmaceutical Sciences, Texas Tech University HSC, Amarillo, TX.*

Combined highly active antiretroviral therapy (HAART) is strongly associated with severe metabolic abnormalities leading to increased incidence of type 2 diabetes in HIV-infected patients. Given the evidence that hyperglycemia inflicts blood-brain barrier (BBB) endothelial dysfunction through elevated oxidative stress, we hypothesized that antiretroviral drug-induced BBB endothelial toxicity would be exacerbated in a diabetic landscape. In this study, we used a well-established human BBB endothelial cell line (hCMEC/D3) to evaluate the effects of zidovudine (AZT)/indinavir (IDV) combination on mitochondrial function in presence or absence of hyperglycemia. Briefly, confluent hCMEC/D3 cell monolayers were exposed to normal (5.5mM) or hyperglycemic (25mM) concentrations of D-glucose in media for 24-72 h alone or with concomitant exposure to AZT (5μM)/IDV (10μM) combination. Total cellular (DCFH2-DA) and mitochondria-specific (MitoSOX-red) ROS levels were determined by flow cytometry in addition to cell viability and mitochondrial membrane potential (ΔΨm) by ATP and JC-1 assays, respectively. Although, hyperglycemia or AZT/IDV alone did not affect the cell viability, concomitant exposure to hyperglycemia and AZT/IDV significantly decreased total ATP levels in hCMEC/D3 cells with progression of time. Hyperglycemia for 24h exacerbated AZT/IDV-induced increase in total cellular ROS levels (compared to hyperglycemia or AZT/IDV alone). Similarly, a significant loss of ΔΨm associated with increased mitochondria-specific ROS following AZT/IDV was amplified by hyperglycemia. Overall, our data indicate that hyperglycemia exacerbates mitochondrial toxicity of certain antiretroviral drug combinations by increased oxidative stress, possibly leading to potential BBB damage. Thus, our study emphasizes a critical need for safety evaluation of HAART regimen in diabetic patients infected with HIV-1.

1622 Cardiovascular and Pulmonary Safety Pharmacology Evaluation of 2 DG in Dog

M. A. Hawk. *Safety Pharmacology, Battelle, West Jefferson, OH.*

Background: 2-Deoxy-D-Glucose (2-DG) is a potential anticonvulsant and disease-modifying agent for epilepsy patients. Methods: We evaluated the cardiovascular and respiratory effects of 2-deoxy-D-glucose (2DG) in surgically implanted radiotelemetered Beagle dogs (n=4/sex) following a single gavage dose of either vehicle, 5, 30, or 90 mg/kg of 2DG using Latin-square crossover study design. There was at least 72 hours (±2 hours) of washout separating each dosing. The parameters evaluated included: cardiovascular function (e.g., systolic, diastolic, and mean blood pressures, heart rate, dP/dtmax and dP/dtmin), body temperature, electrocardiogram (ECG), respiratory function, clinical observations, and body weights. Respiratory data were collected while the dogs were in restraint slings (at least 30 minutes prior to dosing to 240 minutes post-dose) and telemetry data were collected both in restraint slings (at least 30 minutes prior to dosing to 240 minutes post-dose) and unrestrained in their respective home cage (approximately 5 to 48 hours post-dose). Data were analyzed for statistically significant differences as compared to the vehicle data. Results: 2DG-related decreases (maximum percent change from vehicle) occurred in the 90 mg/kg dose group as compared to vehicle for systolic (8.6%), diastolic (9.0%) and mean systemic arterial blood pressures (9.2%). These decreases occurred between 60 and 190 minutes post-dose while the animals were in their slings. Although these changes were significant when compared to vehicle, the changes observed were decreased only to the levels of the in-cage baseline blood pressure values. There were no 2DG-related changes for in-sling ECG, body temperature and respiratory data as well as for all the cardiovascular (hemodynamic and heart rate) and body temperature data in-cage for the 90 mg/kg group as compared to vehicle. Conclusions: There were no 2DG-related changes observed for any cardiovascular, body temperature or respiratory parameters for the 30 and 5 mg/kg groups during the study as compared to vehicle. With the subtle decreases in systemic blood pressure, all doses of 2DG appeared to be well tolerated for this cardiovascular and respiratory study in dogs.

1623 Structural Analysis of Pharmacological Co-Crystals of Furosemide

B. K. London[2], M. O. Claville[3], S. Babu[3], F. R. Fronczek[1] and R. M. Uppu[2]. [1]*Chemistry, Louisiana State University, Baton Rouge, LA;* [2]*Environmental Toxicology PhD Program, Southern University and A&M College, Baton Rouge, LA and* [3]*School of Science, Hampton University, Hampton, VA.*

Previously, we reported the synthesis and crystal structure of pharmaceutical drug furosemide with m-chlorobenzoic acid (The Toxicologist 2014, PS525, 136). Initially, it was thought the crystals were of m-chlorobenzoic acid co-crystallized with furosemide; a detailed structure analysis however revealed that it has a cationic structure of nonahydrated disodium (II) co-crystallized with anions of furosemide and m-chlorobenzoic acid. In this study, we have undertaken a detailed structural analysis highlighting the secondary features of the co-crystals. In the title compound, [Na2(H2O)9](C7H4ClO2) C12H10ClN2O5S) {systematic name: catena-poly[[[triaquasodium(I)]-di--aqua-[triaquasodium(I)]-μ-aqua] 3-chlorobenzoate 4-chloro-2-[(furan-2-ylmethyl) amino]-5-sulfamoylbenzoate]}, both m-chlorobenzoic acid and furosemide exist with deprotonated carboxylates, and the sodium cations and water molecules exist in chains with stoichiometry [Na2(OH2)9]2+ that propagate in the [110] direction. Each of these two independent Na+ ions are coordinated by three monodentate water molecules, two double-water bridges, and one single-water bridge. There is considerable cross-linking between the [Na2(OH2)9]2+ chains and to furosemide sulfonamide and carboxylate by intermolecular O–H..O hydrogen bonds. All hydrogen-bond donors participate in a complex two-dimensional array parallel to the ab plane. The furosemide NH group donates an intramolecular hydrogen bond to the carboxylate group, and the furosemide NH2 group donates an intramolecular hydrogen bond to the Cl atom and an intermolecular one to the m-chlorobenzoate O atom. The plethora of hydrogen-bond donors on the cation/water chain leads to many large rings, up to graph set R44(24), involving two chains and two furosemide anions. The m-chlorobenzoate is involved in only one R22(8) ring, with two water molecules cis-coordinated to Na. The furan O atom is not hydrogen bonded. Pharmaceutical co-crystals as presently described here can have several applications in terms of drug design especially in the context of drug stability and bioavailability in addition to improved manufacturing processes [Supported by NSF grants HRD-1043316 and CHE-1230357, and the US DoE grant PO31B040030 (Title III Part B, Strengthening HBGI programs)].

1624 Nonclinical Safety Studies of *Clostridium difficile* Toxoid Vaccine in Rabbit

G. Ravel[3], A. Caron[2], F. Spezia[1], L. Quemeneur[3], S. Keech[3] and P. Pietrobon[4]. [1]*CiToxLAB, Evreux, France;* [2]*Sanofi, Alfortville, France;* [3]*Sanofi Pasteur, Marcy l'Etoile, France and* [4]*Sanofi Pasteur, Swiftwater, PA.*

Clostridium difficile is a gram-positive, spore-forming anaerobic bacterium that causes diseases ranging from mild self-limited diarrhea to life-threatening pseudomembranous colitis. To prevent primary *C. difficile* infections, Sanofi Pasteur is developing a *C. difficile* Toxoid Vaccine composed of highly purified *C. difficile* toxoids A and B adjuvanted with aluminum hydroxide. The preclinical safety development of the candidate vaccine included a repeat-dose toxicity study and a developmental and reproductive toxicity (DART) study. The repeat-dose study evaluated the systemic and local toxicity of the vaccine in New Zealand White rabbits given one human dose of the vaccine on 4 occasions. The assessment included clinical observations, body temperature, bodyweight, food consumption, clinical pathology, immunogenicity and post-mortem examinations including histopathology. There were no toxicological findings other than limited local reactions at the injection site, which are anticipated with an aluminum-adjuvanted vaccine. The DART study investigated the potential risk for women of childbearing potential and their offspring. Female rabbits were given one human dose of the vaccine on 2 occasions before mating and on 3 occasions during gestation. The assessment included clinical observations, bodyweight, food consumption and immunogenicity. A subset of females was submitted for hysterectomy examination, the remaining females were allowed to deliver and the litters were examined up to weaning. There was no maternal toxicity and no adverse effects on embryo-fetal development or early post-natal development in the rabbits. In both studies, the immunogenicity data demonstrated exposure and immune response to the vaccine. The two toxicology studies showed that there is no evidence of toxicological concern with the *C. difficile* Toxoid Vaccine and therefore support the clinical development of the vaccine candidate.

1625 An Inhalation Safety Assessment Model: A Method for Delivering Test Articles via the Pulmonary Artery

B. M. Roche, R. B. Borders, J. Kieper, K. Landis, N. Stewart, M. Sexton and J. Herrada. *Pharmacology and Discovery Services, WIL Research, Ashland, OH.*

Determining the clinical route of administration is an important component when constructing a drug development plan. The route of administration needs to consider not only the pharmacokinetic profile but also the ability of the patient to self-administer the drug, as well as, if the patient considers the dosing route as painful and/or inconvenient. Inhalation formulations are increasing related to this route having a similar PK profile to intravenous, the ability to self-administer is high (spanning a large age range), is less painful compared to injections, and is perceived as a convenient method for drug delivery. Unfortunately, nonclinical species utilized in safety studies, dosed via the inhalation route, are unable to control deposition of dose, resulting in either estimating the dose or correlating the systemic exposure values to an expected inhaled dose. By directly administering drug into the pulmonary artery (PA), the cardio-pulmonary vascular exposure would be expected to model the theoretical immediate bioavailability of an inhaled deep lung dose while systemic exposures would mimic the intravenous route (with the exception of higher initial cardiac exposure). The objective of this study was to evaluate the effects of phenylephrine and diltiazem on PA pressure when administration via the PA in male beagle dogs under the anesthetized and conscious state. Telemetry instrumented dogs were anesthetized (isoflurane), a Swan-Ganz catheter placed in the PA and dosed with 0.01 mg/kg phenylephrine and 0.02 mg/kg diltiazem (at 1 mL/kg) via slow push (30 seconds) with a washout period between doses. The next day, the dosing was repeated in the conscious condition. Phenylephrine increased PA pressure (~30%), arterial blood pressure and reduced heart rate (~20%). Diltiazem decreased PA pressure (~10%), arterial blood pressure and heart rate. The magnitude of blood pressure change was slightly blunted in the anesthetized condition. In conclusion, dosing via the PA mimics a deep lung exposure while maintaining the ability to detect direct effects of vasoconstriction or vasodilatation of the PA.

1626 Toxicity and Pharmacokinetics of ZMapp™, a Promising Ebola Therapeutic, in Rats

J. P. Bakke[2], M. T. Harrison[2], K. Matsuyama[2], T. Tran[2], K. Altera[2], A. Montano[2], M. B. Brennan[1], T. J. Nyhuis[1], L. Zeitlin[1] and J. Mirsalis[2]. *[1]Mapp Pharmaceuticals, San Diego, CA and [2]Biosciences Division, SRI International, Menlo Park, CA.*

ZMapp™ is a cocktail of three monoclonal antibodies against the surface glycoprotein of the Ebola virus (EBOV) that was used to treat patients during the 2014 EBOV disease outbreak under expanded access provisions prior to its approval for clinical testing. We conducted a safety study of ZMapp in Sprague-Dawley rats to support initiation of clinical trials. Rats (15/sex) were given a vehicle control, 50, or 100 mg/kg ZMapp intravenously on Days 1, 3, 7, 11, 16 and 21. Additional rats (9/sex) were included in the ZMapp groups for toxicokinetic analysis. Ten/sex were necropsied on Day 22 and 5/sex on Day 70. Peak serum concentrations were very high for all three antibodies, ranging from 294-564 µg/ml at 50 mg/kg, and 675-926 µg/ml at 100 mg/kg. Half-life for the three antibodies ranged from 9.0 - 15.4 days. Aside from a minimal antibody titer in one animal, no immunogenicity was observed in any of the samples. There were no changes in mortality, clinical observations, body weights, food consumption, hematology, urinalysis or ophthalmology parameters that were considered related to ZMapp treatment. Modest increases in organ weights were seen in adrenal glands and spleen, but there were no microscopic pathology changes in either organ. Modest changes in several liver biomarkers and increases in liver weights were also observed. These findings are consistent with hepatocyte hydropic degeneration that was minimal-to-moderate in severity and was present at both 50 and 100 mg/kg. Hepatocyte hypertrophy, increased mitotic activity in hepatocytes, disorganization of hepatic architecture, and hepatocyte apoptosis were also observed at both dose levels. All the hepatic effects resolved by the end of the recovery period. Based on these results, the maximum tolerated dose (MTD) of ZMapp is considered to be at least 100 mg/kg for up to six injections over a 21-day period. The no observed adverse effect level (NOAEL) cannot be determined, but it is considered to be less than 50 mg/kg. The effects on the liver are mild, similar to those seen for other marketed pharmaceuticals, and resolve following treatment, and are therefore not considered to be clinically dose limiting. (Supported by NIAID Contract HHSN2722011000221)

1627 Automated Blood Collection for Arterial Blood Gas Sampling in Unrestrained Monkeys

D. Hopper[2], T. Riggs[2], M. Shelton[2], C. Kreilein[2], N. Suttles[2], S. Kurtz[2], B. Kemmerling[2], L. Kinter[1], K. Bigge[2] and P. Kruzich[2]. *[1]GLP Scientific Consulting, Unionville, PA and [2]Toxicology, BASI, Mount Vernon, IN.*

Measuring the impact of drug-induced changes in respiratory function and acid-base status requires serial monitoring of blood pH, pCO2, PO2, and HCO3. Blood sampling during standard cardiovascular radio-telemetry studies is less than optimal due to repeated disturbance of subjects. This study assessed the feasibility of serially collecting respiratory blood gases, pH, and HCO3 in monkeys with automated blood collection, and detecting acute acidosis and alkalosis in conscious monkeys under cage conditions similar to radio-telemetry studies. Four tethered cynomolgus macaques (2/sex) were administered IV infusions of saline (0.75 mL/kg bolus plus 0.125 mL/kg/min for 20 min), NH4CL (250 mg/kg over 20 min), HCO3 (1680 mg/kg over 20 min), and remifentanil (12 µg/kg bolus plus 2 µg/kg /min for 20 min) in a modified Latin Square design wherein each animal received each test article on different days. Serial carotid arterial blood samples (400 µL) were withdrawn by programmed automated blood collection (Culex-L ABC) at baseline, then from 5 minutes to 360 minutes post-infusion via a vascular access port. Samples were then analyzed for pCO2, pO2, pH, and HCO3 (RapidPoint 405 Analyzer).. Baselines values for all parameters were within normal ranges. NH4Cl produced metabolic acidosis (pH -0.2 units, HCO3 -50% by 5 min) followed by compensatory respiratory alkalosis. NaHCO3 produced rapid metabolic alkalosis (pH +0.25 units, HCO3 +100% by 5 min) followed by compensatory respiratory acidosis. Remifentanil produced rapid respiratory acidosis (pH -0.21 units, HCO3 -15% by 5 min) followed by recovery with dissipation of respiratory depression. This study demonstrated the feasibility to collect serial respiratory blood gas, pH and HCO3 concentrations under experimental conditions compatible with cardiovascular radio-telemetry, and thereby to combine ICH S7A core battery respiratory and cardiovascular function assessments within a single study while supporting the 3R's of animal research by decreasing animal use.

1628 A Comparison of Dry Powder Inhalation Setups Used in Nonclinical Testing

M. Freke and A. Viau. *Charles River, Montreal, QC, Canada.* Sponsor: M. Vezina.

Although it is not critical to use the intended clinical device when generating aerosols as part of a nonclinical testing program, it is critical that a suitable setup be selected. The setup has to be able to have the ability to generate stable aerosols sufficient enough to expose the Test System to multiples of the clinical dose required to prove safety and it has to generate an aerosol that can be inhaled by the Test System. With the therapeutic agent often being in limited supply, these criteria must be met using the most efficient setup possible. The purpose of the investigation was to directly compare several aerosol generation setups for their ability to achieve a target aerosol, the spatial and temporal homogeneity of the generated aerosol and the resultant mass transfer efficiency. Two aerosol generation devices were selected, the commercially available PALAS® Rotating Brush Generator (RBG) series 1000 and a device developed at Charles River Montreal (CR-MTL). Aerosols were generated on multiple occasions using sodium bicarbonate into a flow-through or flow-past chamber at target aerosols ranging from 0.1 to 1.0 mg/L. Gravimetric sampling techniques were employed to determine the achieved aerosol concentration at various locations about each flow-past and flow-through chamber; with means, standard deviations and correlation coefficients calculated along with system efficiency for each. Both setups/combinations had the ability to achieve a homogeneous low aerosol concentration (%C.V of <15) at or around 0.1 mg/L, with comparable system efficiencies (5 to 10%). Regardless of chamber type, the RBG was not able to maintain a stable aerosol concentration at the targeted 1.0 mg/L. Careful consideration should be given to the selection of a suitable chamber and dry powder aerosol generation system combination for use on nonclinical inhalation safety programs. It was concluded that any of the combinations tested were suitable for use when targeting a total dry powder aerosol at or around 0.1 mg/L; however, the RBG may be less optimal when targeting higher concentrations (i.e., 1.0 mg/L).

1629 *In Vivo* Safety Testing of Biosimilar Drugs–Recent Experiences

P. Baldrick. *Regulatory Strategy, Covance Laboratories, Harrogate, United Kingdom*. Sponsor: P. Sausen.

Key in the development of a biosimilar ("follow on") molecule, whether a monoclonal antibody (Mab) or a non-Mab protein, is biological activity/nonclinical comparability to the marketed (innovator) drug. Although some regulatory guidelines are encouraging little or no *in vivo* testing, especially in the form of toxicity testing, currently a common default is to perform at least one toxicology and/or one pharmacokinetic (PK) study to assess if any different findings occur for in-life, clinical pathology and histopathological parameters or exposure between biosimilar and innovator drugs. In order to examine what findings are seen, an exercise was performed in which the results of such testing were evaluated. It was found that a snapshot of 10 PK comparison studies performed in the cynomolgus monkey across 4 Mab classes from 2009-2014 showed similar exposure in all cases. A snapshot of 15 toxicology comparison studies with 5 Mab classes performed in the same species and over the same period showed no new/unexpected findings (with class effects seen in a comparable manner for some of the Mabs) and drug exposure measurement giving similar values in all cases. Similarly, from a snapshot of toxicology testing of 7 non-Mab biosimilars in the rat from 2011-2013, results showed no differences in study endpoints and similar drug exposure level for biosimilar and innovator drugs. Overall, although this work highlights utility of some *in vivo* testing (notably in the form of stand-alone PK testing) to confirm similar exposure between the 2 molecules tested, it is unclear what clear benefit can be gained from toxicity testing, especially if comparability has been demonstrated from *in vitro* biological activity testing and in the case of primate use, the lack of statistical power to formally assess biosimilarity due to small numbers of animals used.

1630 Development and Use of *In Vitro* Alternatives to Animal Testing by the Pharmaceutical Industry 1980–2013

R. Roberts[1], J.-Y. Goh[3], R. Weaver[2], N. Platt[3] and L. Dixon[3]. [1]*ApconiX, Alderley Edge, United Kingdom;* [2]*Servier, Suresnes, France and* [3]*The Association of the British Pharmaceutical Industry, London, United Kingdom.*

We examined the use of *in vitro* (including *in silico*) techniques in preclinical safety testing by the pharmaceutical industry between 1980 and 2013 to determine patterns, drivers and challenges in uptake. Data were collected via a survey sent to the Association of the British Pharmaceutical Industry (ABPI) member companies from the Nonclinical and Biological Discovery Expert Network (NaBDEN) requesting the number of compounds screened using *in vitro* and *in silico* tests at 5-year intervals between 1980 and 2005 then yearly from 2008 onwards. A utility score from 1 (poor) to 5 (excellent) for each assay was also requested. Four pharmaceutical companies and 3 contract research organisations (CROs) responded to the survey, providing >895000 data points across all years and all assays. Overall, there was a steady increase in the use of *in vitro* tests by the pharmaceutical industry between 1980 and 2013; indeed >20% of all *in vitro* tests reported were conducted in the last year of the survey window (2013) and >70% of all *in vitro* tests reported were conducted since 2010. Use of *in vitro* tests peaked at >190 000 tests per annum in 2012; >99% of this usage was in the three main areas reported of ADME, safety pharmacology and genotoxicity. Trends and step changes in uptake were most notable in the three main areas of ADME, safety pharmacology and genotoxicity and may be explained by the timing of adoption of the relevant International Committee on Harmonisation (ICH) guidelines. Trends in uptake may also be explained by perceptions of utility where scores varied from poor (Eye Irritation - flourescein leakage) to excellent (Genotoxicity - Ames and Skin irritation - EpiSkin/Epiderm). In summary, the data show a large increase and a continuing upwards trend in development and adoption of *in vitro* alternatives to animal testing in pharmaceutical drug development providing new opportunities to improve success rates coupled with a strong commitment to the 3Rs.

1631 Identification of Leukocyte Subsets in Cynomolgus Monkey Spleen Using Flow Cytometry

C. S. Eberle, D. Trani, R. Chen, M. Perpetua, Y. Q. Xiao and G. Bannish. *Princeton Research Center, Envigo, East Millstone, NJ.* Sponsor: G. Bannish.

To evaluate major immune cells in cynomolgus monkey spleen using flow cytometry, we developed a single, eight-color panel. Immunophenotyping of lymphocytes, monocytes, natural killer (NK) cells, B cells, T cells and T cell subsets was validated following a fit-for-purpose approach. Commercially available fluorochrome-conjugated antibodies were utilized, including CD45-V450, CD3-AF488, CD4-PE-Cy7, CD8-V500, CD14-PerCP-Cy5.5, CD16-PE, CD20-APC-H7, and CD49d-APC. Single cell suspensions of spleen were freshly prepared, incubated with erythrocyte lysing buffer, washed and tested for viable cell concentration using 0.1% (w/v) trypan blue. Diluted spleen suspensions were blocked with mouse serum, stained with CD45/CD3/CD4/CD8/CD14/CD16/CD20/CD49d antibodies, then washed, re-suspended and fixed. Lymphocytes were delineated by side scatter (SSC) properties and CD45 expression to eliminate debris and doublets. CD14 fluorescence was plotted against side scatter to identify monocytes as CD14+/SSC low cluster. Total T cells and B cells were gated from lymphocytes based on expression of CD3 or CD20 antigens, respectively. Within total T cells, T helper cells and cytotoxic T lymphocytes were clearly separated by their CD4 or CD8 binding, respectively. Furthermore, NK cells were defined as population bearing a CD3-CD16+ phenotype. Percentages of the leukocyte subsets were reported for each phenotype. Intra-assay precision was found below 20% CV for all cellular subsets. A relative difference of <30% for all gated percentages demonstrated low variance between different analysts. Spleen samples were stable up to 24h post-collection and up to 24h post-fixation when kept refrigerated at 2–8°C. Overall, this immunophenotyping assay passed pre-defined performance criteria regarding precision, stability and robustness. Splenic analysis using this validated method can be routinely used in preclinical safety evaluation. It complements assessment of potential toxicity of a test article on leukocyte subsets in cynomolgus monkeys.

1632 Retrospective Study of Clinical Pathology Changes in Sick or Moribund Animals

L. Ramaiah. *Safety Assessment, Envigo, East Millstone, NJ.*

In drug safety testing, moribund animals are euthanized early to reduce the duration of pain and distress prior to death and to avoid post-mortem autolysis of tissues. Unscheduled decedents generally undergo a limited battery of evaluations to identify the cause of moribundity and to evaluate test article-related findings at lethal doses. Blood for clinical pathology may be collected from sick animals, ideally prior to moribundity, to inform on physiologic state and prognosis and to direct the decision of whether to pursue palliative care, modify dosing regimens, or humanely euthanize. However, clinical pathology has limited value for identifying test article-related effects in severely sick or moribund animals, since results are either too variable or primarily due to their debilitated state. Clinical pathology changes classically associated with moribund state are well described and are generally attributed to poor sample quality (due to difficult phlebotomy), stress, dehydration, tissue hypoxia, hemodynamic shock or agonal changes. They can involve essentially any endpoint included on a routine clinical pathology panel. Conversely, the clinical pathology findings could potentially represent direct and adverse test article-related effects that are responsible for the deteriorating clinical condition of the animal. This raises the question of the value of blood collection from sick or moribund animals and leaves investigators struggling with decisions regarding whether to collect blood for clinical pathology from these animals and the interpretation of data obtained from these collections. To more fully characterize clinical pathology changes associated with severe illness or moribund state, we have reviewed data from mouse, rat, rabbit, minipig, dog, and monkey toxicity studies performed at Envigo from 2009 to 2015. For each species, clinical pathology results from unscheduled decedents are correlated with specific in-life (clinical signs, body weight, food consumption), organ weight, macroscopic, and microscopic findings to document their frequency and likely cause (including adverse test article-related effects responsible for moribund state). Results presented in this retrospective investigation will provide investigators with specific examples and guidance for the value of collections and interpretation of clinical pathology changes in sick animals.

1633 Long Term Intrathecal Bolus Administration Studies in Cynomolgus Monkeys: Expected Background Findings, Procedure-Related Observations and Recommended Procedures

F. Ludwig, S. Korte and M. Niehoff. *Covance Preclinical Services GmbH, 48165 Muenster, Germany.*

Nonclinical studies in cynomolgus monkeys involving direct lumbar CSF administration under sedated conditions are fundamentally different than studies with more conventional routes and several important points should be considered with their design especially at time of study report generation. Preclinical safety studies on Central Nervous System (CNS) administered compounds must differentiate between the effects of the compound, the delivery technique, side effects caused by e.g. dose volume and sedation, and background findings unrelated to treatment. In this context, only a limited amount of data is available citing the background clinical signs vs treatment-related indications. Conducting long term (up to 53 weeks) cynomolgus monkey studies (*Macaca fascicularis* n≥240, Asian origin, 10 months to 4 years) under European housing conditions, animals were dosed intrathecally only in long intervals, whilst physical examinations were conducted once weekly, with animals in hand. Occurrence and severity from background findings such as injuries, tail lesions, tooth fracture, genital region findings, swellings, hair loss, diarrhea, reduced food consumption, blue eye lids, nasal discharge, vomiting, crusted eyes, squamous skin, hair loss, mammillae findings, thin appearance, low body score and discolored skin were similar to same duration oral gavage or subcutaneous dosing. Sedation of the animals (s-ketamine and meditomidine, atipamezole or propofol) and lumbar bolus dosing of up to 2 mL (after withdrawal of equal amount) resulted in transient neurological findings, such as temporarily unilateral or bilateral, reduced or non-detectable patellar reflex in controls animals. In addition, single cases of ataxia, lameness of legs or/and slight tremor were present (up to 4 hours) and considered to be related to the sedation. In the cited cases, warming lamp and sodium chloride supportive treatment were the recommended procedures. A diazepam derivate would be applied if severe post dose findings would be observed, such as spasms.

1634 Assessing the Changing Landscape of Microsampling Use through a Global Cross-Company Initiative

J. Edwards, A. Bottomley and K. Chapman. *NC3Rs, London, United Kingdom.* Sponsor: N. Burden.

By taking smaller sample volumes, microsampling can increase the scientific value of data obtained from rodent studies during drug and chemical discovery and development, and can enable multiple endpoints to be investigated in the same animal. It also provides the opportunity to reduce the number of animals used. Ultimately, the long term goal of the industry is to sample main study animals to link toxicity to exposure in the same animal and maximise the scientific benefits. To assess the uptake and use of microsampling techniques, and to understand the barriers to the wider uptake of microsampling approaches, the NC3Rs manage an expert microsampling user group and have carried out two surveys of this group in 2013 and 2015. Two main barriers to the uptake of microsampling have been identified in the surveys. The first is the concern that taking microsamples may impact on routinely measured toxicology endpoints and the second is related to practicalities associated with implementing a new technique. Despite these barriers the surveys show that there has been an increase in the number of companies that carry out microsampling over the last two years particularly in regulatory safety pharmacology (4-fold increase) and toxicology (2.5-fold increase) studies. Microsampling in these cases has not had an impact on regulatory acceptance. The majority of respondents report an impact of 21-40% decrease in the number of rodents used in these studies with some reporting a reduction of more than 60%. The results of the survey and case studies of successful implementation of microsampling from industry will be presented. The microsampling user group has also shared data and experience on the effect of microsampling on common toxicological endpoints. These data and the information from the survey has fed into ICH S3 Q&A and also exists as a practical web resource which will provide support to potential users and increase confidence in microsampling approaches.

1635 Inhibition of Solute Carrier (SLC) Transporters Assayed in Corning® Transportocells with Radiolabelled Probe Substrates Synthesised In-House

C. Cantrill, J. Lewis, V. Wilson, D. Hendry and M. Hall. *Quotient Bioresearch Ltd, Rushden, United Kingdom.* Sponsor: P. Atterson.

Drug-drug interactions (DDIs) mediated by SLC transporters have been increasingly identified as a key determinant of pharmacokinetic interactions. Consequently, regulatory authorities require an assessment of the potential of a candidate drug to inhibit at least seven SLC transporters (Organic Anion-Transporting Polypeptides 1B1, 1B3 (OATP1B1, OATP1B3), Organic Anion Transporters 1, 3 (OAT1, OAT3), Organic Cation Transporter 2 (OCT2) and Multidrug and Toxin Extrusion proteins 1, 2-K (MATE1, MATE2-K)). The purpose of this study was to establish the use of Corning® TransportoCells as a system for SLC DDI assessment at Quotient Bioresearch using radiolabelled probe substrates synthesised in-house. Initially, assays of nine SLC transporters (OATP1B1, OATP1B3, OATP1A2, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K) were validated, using radiolabelled probe substrates ([3H]p-aminohippuric acid, [3H]estrone 3-sulphate, [3H]estradiol 17β-glucuronide, [14C]metformin hydrochloride). Assay sensitivity to inhibition was assessed using established inhibitors (probenecid, decynium-22, rifampicin, pyrimethamine). All radiolabelled probe substrates were a minimum of 97% purity for use. The inhibitory profiles generated with the radiolabelled substrates and positive control inhibitors were consistent with IC50s presented by Corning® and with information within the literature. IC50 values obtained ranged from 0.13 to 13 μM across all nine transporters assessed, including 1.99 μM for rifampicin against OATP1B3, 9.40 μM for probenecid against OAT1, 4.08 μM for decynium-22 against OCT2 and 0.13 μM for pyrimethamine against MATE1. In conclusion, robust methods to assess inhibitory DDI risk in nine individual SLC transporters have been developed at Quotient Bioresearch. These assays will be used in the future assessment of the potential of candidate drugs to inhibit SLC transporters and hence the risk of clinical DDIs.

1636 Abstract Withdrawn by Author.

1637 Bridging Repeat Dose Toxicity Studies in Adult and Juvenile Rats for a Dopamine Receptor Antagonist, Molindone

G. Krishna, S. Goel and G. Gopalakrishnan. *Supernus Pharmaceuticals Inc, Rockville, MD.*

Dopamine D2 and D5 receptor antagonist, molindone, was originally developed for the management of schizophrenia. An extended-release formulation of molindone is currently in late-stage development for impulsive aggression in patients with attention-deficit/hyperactivity disorder and has received fast-track designation from the Food and Drug Administration (FDA). The purpose of these studies was to bridge the data gaps by the original innovator in light of the current indication and regulatory standards. To this end, a variety of preclinical studies were identified in consultation with FDA. Repeat dose toxicology studies in adult and juvenile rats are reported here. For adult rats, molindone was dosed po at 0, 5, 20 or 60 mg/kg/day for 3 months and standard toxicological parameters evaluated. In juvenile rats, routine toxicity and effects on reproductive and neurobehavioral development, plasma insulin-like growth factor 1 (IGF-1), bone length and density were evaluated following oral dosing at 0, 25, 50, or 75 mg/kg/day for 8 weeks starting at postnatal day 23, continuing to adulthood. Further, the reversibility, persistence, or delayed effects following a 6 week nondosing period were measured. In adult rats, molindone caused behavior-related clinical observations including decreased activity and partial closure of the eyes. Dose-related decreases in body weight gain (BWG) and food consumption (FC) in males and mammary gland hypertrophy/hyperplasia in females were noted. Changes in reproductive tissues in males (suppurative exudate in the prostate) and females (vaginal and cervical epithelial mucification, hypertrophy of ovarian corpora lutea, and hypertrophy or hyperplasia of ovarian interstitial cells) were attributed to drug-related pharmacologic effects of prolactin, and are considered not relevant to humans due to species specific differences in hormonal regulation of reproduction. In juvenile rats, no observed adverse effect level (NOAEL) for general toxicity was 25 mg/kg/day and the NOAEL for reproductive toxicity was 75 mg/kg/day. Most of the changes noted at ≥ 50 mg/kg/day (sexual maturation, IGF, bone length and density) were related to lower FC resulting in lower BWG. All data considered together, it was concluded that the changes seen in these studies were comparable to those reported by the original innovator, bridged the data gaps to current standards and supported the safety profile of molindone.

1638 BTK Small Molecule Inhibitors Induce Pancreatic Toxicity in Sprague-Dawley Rats

R. Erickson[1], L. Schutt[1], J. Tarrant[1], M. McDowell[1], H. Wong[2], L. Liu[1], J. Crawford[1], R. Carano[1], S.-C. Lewin-Koh[1], K. Staflin[1], E. Murrary[1], W. Kennedy[1], U. Klein[1], D. Diaz[1], E. Harstad[1] , D. Danilenko[1], W. Young[1], D. Dambach[1] and D. Misner[1]. *[1]Genentech, Inc., South San Francisco, CA and [2]University of Vancouver B.C., Vancouver, BC.*

Bruton's tyrosine kinase (BTK) is a member of the Tec family of cytoplasmic tyrosine kinases involved in B cell and myeloid cell signaling. Inhibitors of BTK are being developed to treat a variety of diseases mediated by these cell populations, including a spectrum of hematological cancers as well as autoimmune diseases. Ibrutinib, a highly effective treatment for B cell malignancies, is an FDA-approved, covalent and irreversible BTK inhibitor that is generally well tolerated; the most common adverse events reported are diarrhea, fatigue, and upper respiratory infections. GDC-0853 is a selective, reversible, and orally administered BTK inhibitor being developed as an investigational agent for the treatment of rheumatoid arthritis. GDC-0853 administration to 8-10 week old Sprague-Dawley (SD) rats for ≥ 7 days was associated with pancreatic histopathology consisting of multifocal islet/peri islet hemorrhage, inflammation, fibrosis, and pigmented macrophages with adjacent lobular exocrine acinar cell atrophy and degeneration. Similar findings did not occur in mice, dogs, or in Han-Wistar (WH) rats at exposures that far exceeded the SD rat LOAEL for pancreas histopathology. A timecourse study in SD rats revealed that the acute stage, which emerges between 4 and 7 daily doses, is histologically similar to a spontaneously occurring lesion described in aging SD rats, suggesting that GDC-0853 could be exacerbating a background finding in younger animals. The pancreas lesions were associated with mild dysregulation of glucose homeostasis following a PO or IV glucose challenge, but not associated with changes in other commonly used biomarkers of endocrine and exocrine function. The lesion was not detectable via Doppler ultrasound or CT imaging techniques in SD rats administered GDC-0853 for 14 days. Similar lesions were observed in SD rats administered other highly selective, structurally related BTK inhibitors and also with ibrutinib (structurally distinct). The totality of the data suggest that these pancreatic lesions in SD rats are likely a class effect of BTK inhibitors, which may be exacerbating a background lesion in this species/strain.

1639 Diagnostic Tools to Enhance Long Term Intrathecal Delivery Studies in Non-Human Primates

S. Korte, M. Niehoff and F. Ludwig. *Covance Preclinical Services GmbH, 48165 Muenster, Germany.*

While direct Central Nervous System (CNS) administration is likely to serve as an emerging delivery route in treatment of neurodegenerative disorders, preclinical development of potential therapeutics in non-human primates faces a number of challenges. Preclinical safety studies on CNS administered compounds must differentiate between the effects of the compound, the delivery technique, side effects caused by e.g. dose volume and sedation, and background findings, unrelated to treatment. In this context, the publication cites a variety of diagnostical "add-on" tools such as modified Irwin test, neurological screening and Ethovision (Noldus Information Technology). In overall five studies n≥240 cynomolgus monkeys (Asian origin, up to 4 yrs, for a duration up to 13 weeks) underwent pre-dose and subsequent screening. Hence, physical and neurological examinations were performed in unsedated animals (on at least 5 occasions) during the predose phase and the dosing phase of toxicity studies (predose, 4 and 8 hours postdose). In case of findings that did not recover within 8 hours postdose, additional examinations were performed. Physical examinations included abdominal palpation, eye, ear and nose examination, body temperature, heart, and lung auscultation. Neurological examinations included general sensomotory aspects, cerebral reflexes (pupillary, orbicularis oculi, cornea) and spinal reflexes (patellar, anal). Furthermore, the foot grip reflex (a rod was placed under the foot and the animal should grip this rod) was recorded. In addition a behavioural in-cage observation was conducted and monkeys X vs. Y coordinates were filmed for 30 minutes using Ethovison®. Effects that were related to the intrathecal administration (procedure-related, as also occurring in control animals and not being present before the dosing phase), were either a temporarily absent or reduced patellar tendon reflex (4, 8 and 24 hours after dosing) or the absence of the anal reflex (4 and 8 hours). These findings were all of transient nature.

1640 *In Vivo* Reactivation of Murine Gammaherpesvirus-68 (MHV-68) from Latently Infected Syngeneic Tumor Cells Due to Treatment with Immunomodulators

J. Aligo, K. Brosnan, P. Rafferty and D. Weinstock. *Janssen Research and Development, Spring House, PA.*

Immunosuppressive therapy in humans can cause reactivation of latent herpesvirus infections. Following tissue transplant, Epstein Barr Virus (EBV) reactivation is associated with post-transplant lymphoproliferative disorder (PTLD), which may progress to B cell lymphoma. Murine Gammaherpesvirus-68 (MHV-68) is related to EBV, and is a proposed mouse model of latent EBV infection and EBV-associated lymphoproliferative disease. A murine model of EBV infection may be useful in assessing the risk of herpesvirus reactivation for novel immunotherapeutics. MHV-68 latently infected syngeneic lymphoma cells (A20-HE-2) were injected intravenously into normal immunocompetent (Balb/c) mice and allowed to establish for ~3 weeks to generate high tumor load and high level of latent infection. As CD8+ T-cell immunity is critical for maintenance of herpesvirus latency and tumor immunosurveillance, immunodeficient (SCID/Beige) mice were administered A20-HE-2 cells to demonstrate the effect of profound immunocompromise on virus reactivation. Balb/c mice were treated with T-cell immunomodulators (Cyclosporine A (CsA) or Abatacept). Endpoint assessments were viral reactivation from latency and/or accelerated tumor cell growth. Macroscopic findings at necropsy determined that tumors developed predominantly in liver. Quantitative PCR (qPCR) was used to demonstrate the presence or absence of an MHV-68 lytic gene (RTA). Dual *in situ* hybridization (ISH) / immunohistochemistry (IHC) were developed to examine MHV-68 gene expression and protein production. SCID/Beige mice demonstrated increased tumor load as compared to Balb/c mice, robust viral gene expression, and early mortality. Balb/c mice treated with immunomodulators showed transient MHV-68 recrudescence in liver, spleen, and lungs by qPCR but not visualized by ISH.

1641 Neurological Effects of Chronic Treatment with Fulranumab in Sexually Mature Cynomolgus Monkeys

M. Rocca[1], L. Kuseryk[1], C. Han[1], C. Pendley[1], M. Butt[2] and T. Coogan[1]. *[1]Janssen R & D, Spring House, PA and [2]Tox Path Specialists, Frederick, MD.*

Introduction: Fulranumab, a fully human IgG2 anti-nerve growth factor (NGF) monoclonal antibody, is in clinical development as a biotherapeutic for pain. This study evaluated the effects of fulranumab on the sympathetic nervous system (SNS) in sexually mature cynomolgus monkeys (4/sex/group/necropsy) following subcutaneous (SC) administration of 0.25 or 0.7 mg/kg fulranumab once every four weeks (Q4W) for 28 weeks, and 0.2 or 1 mg/kg once weekly (Q1W) for 28 or 52 weeks. Recovery was evaluated either 13 or 24 weeks following 28 weeks of dosing and 26 weeks following 52 weeks of dosing. The objective was to determine a no observed effect level (NOEL) and/or recovery of effects at exposures at least 5-fold higher than the predicted human clinical exposures. Results: There were no effects of fulranumab treatment on any in-life parameters, gross necropsy findings, and organ weights. Fulranumab did not cause any neuronal death, necrosis, degeneration, or apoptosis in sympathetic ganglia at any time point examined. Following 28 weeks of fulranumab treatment there were no effects on the SNS in the 0.25 mg/kg Q4W group. At all higher doses, 28 weeks of treatment with fulranumab caused a diffuse decrease in neuron size (superior cervical ganglion), observed morphologically and stereologically, with a resulting appearance of increased glial cell density. These effects recovered fully in the 0.2 mg/kg Q1W groups after a 3-month treatment free period. Conclusions: The NOEL for fulranumab administered to cynomolgus monkeys SC Q4W for 28 weeks was 0.25 mg/kg. Systemic exposure at this dosage was 5 fold higher than the 3 mg Q4W clinical exposure. Complete recovery of effects was observed following a 13-week treatment-free period in animals treated with 0.2 mg/kg Q1W for 28 weeks. Exposure at that dosage was 5.5 fold higher than the 9 mg Q4W clinical exposure.

1642 Evaluation of the Internal Exposure to Squalene-Containing Adjuvant Following Intramuscular Injection of H5N1 Influenza Vaccine to Mice

M. A. Tegenge[1], L. S. Von Tungeln[2], R. J. Mitkus[1], S. A. Anderson[1], M. M. Vanlandingham[2], R. A. Forshee[1] and F. A. Beland[2]. *[1]US FDA, Center for Biologics Evaluation and Research, Office of Biostatistics & Epidemiology, Silver Spring, MD and [2]US FDA, National Center for Toxicological Research, Division of Biochemical Toxicology, Jefferson, AR.*

Squalene is a component of oil-in-water emulsion adjuvants used in certain influenza vaccines marketed outside the United States. The pharmacokinetics and internal exposure to squalene-containing adjuvants following intramuscular injection of influenza vaccine remain largely unknown. We evaluated the biodistribution of squalene up to 14 days in mice after intramuscular injection of the emulsion adjuvant (AddaVax™) alone or with H5N1 antigen. Blood concentration-versus- time data were analyzed using non-compartmental and nonlinear mixed-effect modeling methods. At 1h, 55% of the injected dose of squalene was detected in quadriceps muscles and this decreased to 26% at 48 h. Twenty-four hours after the injection about 5%, 1% and 0.6% of the injected dose was detected in inguinal fats, draining lymph nodes and sciatic nerves, respectively. The peak concentration for kidney, brain, spinal cord, bone marrow, or spleen was less than 1% of the injected dose, and H5N1 antigen did not significantly alter the biodistribution of squalene to these tissues. The liver concentration peaked at 3h for the AddaVax injected group (3.4±1.7%) and at 6h for AddaVax+H5N1group (2.2±0.4%) but the area-under-liver- concentration curve was about 16% higher in the presence of H5N1.The area-under-blood-concentration curve (AUC) and peak blood concentration (Cmax) of squalene were slightly higher (~20%) in the presence of H5N1 antigen. A population pharmacokinetic model-based statistical analysis identified body weight and H5N1 antigen as covariates influencing the blood clearance of squalene. The time course of internal exposure to squalene-containing adjuvant in mice will contribute to regulatory benefit-risk analyses of this class of vaccine adjuvants.

1643 Toxicology Evaluation of Drugs Administered via the Intranasal Route

A. Emami, N. S. Patel, J. H. Chang and R. D. Mellon. *Center for Drug Evaluation, US FDA, Silver Spring, MD.*

Recently the interest in intranasal delivery as a non-invasive route of administration for drugs has been increasing. Many different drug products may be administered intranasally for topical, systemic and central nervous system (CNS) effects. The nasal mucosa offers numerous benefits as a target tissue for drug delivery. Nasal drugs for treatment of allergic rhinitis or decongestants benefit from the local effects of this route with low systemic adverse effects. Nasal drugs such as hormone or vitamin replacements take advantage of the rapid systemic absorption by avoiding GI and hepatic metabolism. Intranasal drugs such as pain treatments, migraine drugs, and nicotine replacement benefit from the rapid delivery of drugs directly via the nasal mucosa to the brain and spinal cord with the aim of treating CNS disorders while minimizing systemic exposure. As the need for nasal therapeutic compounds increases, toxicological assessments of these drugs play an important role in human safety. We have outlined the local toxicology evaluation for appropriate histopathological assessment and techniques to support safety during the development of nasal drugs in nonclinical models: 1) Selecting the most appropriate species with comparable nasal cavity to human, 2) Conducting a nonclinical study that closely mimics the intended use with adequate duration of intranasal administration, 3) Evaluation of local tissues in both olfactory epithelium and olfactory bulb with special histological staining procedures to identify neuronal and axonal degeneration/necrosis, 4) Functional assessment of Intranasal drugs on olfaction such as a smell test, if deemed necessary, and 5) Evaluation of GI toxicity as the drug can be swallowed after Intranasal administration.

1644 Non-Clinical Safety Profile of CEA-IL2v and FAP-IL2v Immunocytokines: Does Targeting Impact Toxicity?

F. Crameri[3], S. Weber[3], A. M. Giusti[2], S. Dudal[3], H. P. Grimm[3], D. Tuerck[3], H. Hinton[2], J. Patterson[3], S. Bassett[3], P. Maliver[3], I. Waldhauer[2], A. Freimoser-Grundschober[2], V. Nicolini[2] and C. Klein[2]. *[1]Oncology Discovery & Translational Area, Roche Innovation Center Zuerich, F. Hoffmann-La Roche Ltd, Zuerich, Switzerland; [2]Pharmaceutical Sciences, Roche Innovation Center Zuerich, F. Hoffmann-La Roche Ltd, Zuerich, Switzerland and [3]Roche Pharma Research & Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland.*

IL-2 therapy with Proleukin® (aldesleukin) causes major toxicities and the anti-tumor immunity is compromised by induction of regulatory T cells (Treg) cells and activation-induced cell death (AICD).To overcome these limitations, two different IL-2 immunocytokine variants (IL2v) were designed. Fusion of a single IL-2v moiety to a human IgG1 antibody directed against either Fibroblast Activation Protein (FAP) or Carcinoembryonic antigen (CEA) resulted in two clinical candidates: FAP-IL2v (RG7461) and CEA-IL2v (RG7813). FAP is highly expressed on the cell surface of cancer-associated stroma cells of > 90% of human epithelial malignancies whereas CEA is highly expressed on the cell surface of several tumors. Key feature of these novel IL2v based immunocytokines are: a) preferential targeting of the immunocytokine into the tumor, b) reduced AICD and preferential activation of NK and T cells over Treg cells by abolished binding to IL-2 receptor-alpha (CD25), c) lack of antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) via abolished interaction with Fcγ receptors (FcgRs). This poster summarizes the two different non-clinical safety strategies pursued to support clinical development of both targeted molecules. For CEA-IL2v a surrogate molecule was tested in *in vivo* preclinical toxicology studies, due to the lack of cross-reactivity of the anti-CEA component of the clinical candidate with any toxicology species. In the FAP-IL2v project, the clinical candidate was utilized for the EIH enabling toxicity studies. A comparison of the toxicological profiles of both molecules after repeated i.v. administration in 4 wk GLP studies in Non-Human Primates is provided. Results are also compared to available tissue expression of either CEA or FAP in healthy animals to explore the impact of targeting on IL2v toxicity.

1645 Basmisanil, a Negative Allosteric Modulator of the Gamma-Aminobutyric Acid aα5 Receptor Subtype, Does Not Show Convulsions at Relevant Exposures

A. Rothfuss[2], C. Wandel[1], A. Thomas[2], E. Husar[2], R. Gasser[2], M. Schmitt[2], G. Trube[2], F. Knoflach[2], J. Nöldeke[2] and M.-C. Hernandez[2]. *[1]Clinical Safety, Roche Pharma Development, Basel, Switzerland and [2]Roche Innovation Center Basel, Roche Pharma Research and Early Development, Basel, Switzerland.* Sponsor: L. Mueller.

Basmisanil (RG1662) is a negative allosteric modulators (NAMs) of the gamma-aminobutyric acid, type Aα5 subunit-containing receptor (GABAAα5) and is currently in development for treatment of intellectual disability in Down syndrome (DS). Non-selective GABAA NAMs are known, amongst others, to be associated with a pro-convulsive risk, presumably through interaction with GABAAα1 and α2 subunit-containing receptors. Because epilepsy is a frequent comorbidity in DS, we developed a translational profiling strategy to ensure an absence of pro-convulsivity at therapeutically relevant doses covering the full GABAAα5 occupancy range. *In vitro*, basmisanil was shown to exhibit more than 90 fold binding selectivity for the human GABAA α5 receptor subtype versus GABAA α1, α2, α3, α4 and α6 subunit-containing receptors. At doses shown to improve cognition in preclinical models, basmisanil lacked anxiogenic and proconvulsive potential. Only when basmisanil was given in combination with a sub threshold dose of pentylenetetrazole (PTZ), a potent proconvulsant, were convulsions noted at exposures higher than those needed to fully occupy the GABAAα5 receptor. No convulsions and no EEG alterations were noted in GLP toxicity studies up to supratherapeutic exposures. The discrepancy between PTZ test and GLP toxicology studies may be related to synergistic activity through PTZ in the GABAergic system. A Cmax,free cap to not exceed a ninth of highest Cmax,free in GLP animal studies has been implemented in early clinical trials. The absence of epileptic abnormalities in EEG in early clinical trials supported the inclusion of DS individuals with well controlled epilepsy.

1646 Integrated Arrhythmia Analysis within the DSI Ponemah Data Acquisition and Analysis Program: First Experience with Data Insights

H. Holzgrefe[1], R. Kaiser[1], K. Norton[1], N. Sadekova[1], A. Mehendale[2] and C. Kolin[2]. [1]*Charles River Laboratories, Reno, NV and* [2]*Data Sciences International, St. Paul, MN.*

Unexpected arrhythmias have been associated with the withdrawal, or restricted use, of many pharmaceuticals over the past decades. Due to low incidence and/or underpowered study designs (snap shot analysis), the robust preclinical detection of cardiac arrhythmias remains problematic. Many treatment-related arrhythmias are only confirmed in late development during adequately powered clinical trials. Employing a combination of algorithm and template recognition ECG analysis, DSI (Data Sciences International, St. Paul, Minn) developed a novel arrhythmia analysis module (Data Insights) which rapidly performs beat level scans of digital ECG files (≤ 4 GB) employing species-specific customizable arrhythmia detection algorithms. Employing parallel blinded categorization, 163 ventricular and atrial arrhythmia variants from 38 datasets (dog, cyno, and minipig) demonstrated complete concordance between Data Insights and a veterinary cardiologist. Isolated premature atrial complexes (3/11) constituted the only differences in the parallel analysis. To further test the capabilities of Data Insights in a production setting, 24 h digitized datasets from 3 animals (3 doses and prestudy) in an ascending-dose study with a novel small molecule were blindly analysed for arrhythmia incidence and compared to the the results obtained by a veterinary cardiologist employing extensive (15 min/h) snap shot analysis. Both the automated and manual analyses detected similar low incidences of background arrhythmias (PAC, PVC, 2° AVB) in all animals and treatment groups with the single exception of 1 high dose subject where both methods indicated a clear time and dose-dependent increase in ventricular ectopy which persisted from ~3 to 6 h postdose (peak incidence of 100 PVCs between 3:00 and 3:30 h postdose). Data Insights continuous analysis enabled robust PK/PD modelling of the proarrhythmic events, confirming an unambiguous drug-dependent effect that is beyond the scope of conventional snap shot analysis.

1647 FcRn-Mediated Transport *In Vitro* and Gestation Age Dependent Transfer of Human Immunoglobulins Across Placenta in Timed-Pregnant Guinea Pigs

Y. Xi, M. G. Norton, C. Stuart, L. Zhong, P. Zhang and E. B. Struble. *US FDA, CBER, Silver Spring, MD.*

Introduction: Antibodies and other human biologic drugs that contain the Fc part of the IgG molecule can traverse the placenta when administered during pregnancy. Gaps remain in our understanding of underlying details of FcRn receptor mediated transfer in common laboratory animal species. Methods: We measured the transport of human (h) IgG at the end of pregnancy and at different gestation ages *in vivo* in timed-pregnant guinea pigs. In addition, we evaluated FcRn function *in vitro* using Surface Plasmon Resonance (SPR) and Madin-Darby canine kidney cells (MDCK) that express guinea pig (gp) FcRn. Results: In timed-pregnant guinea pigs the average concentration of hIgG in the fetus increases exponentially with gestation age. *In vitro*, gpFcRn immobilized on a solid surface can bind all human IgG subclasses, hIgG and gpIgG preparations in a similar manner. All engineered hIgG subclasses are internalized by MDCK-gpFcRn cells at significant levels. Discussion: Pregnant guinea pigs exhibit similarities with humans in the degree and timing of transplacental transfer as well as the ability of their FcRn to bind and internalize hIgG *in vitro*. Further studies are needed to guide building appropriate systems for the evaluation of FcRn mediated function of human immunoglobulin therapies. Disclaimer: The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent Agency determination or policy.

1648 Carcinogenic Assessment of Baricitinib in Rats and Mice

M. A. Carfagna and J. M. Sullivan. *Nonclinical Safety Assessment, Eli Lilly and Company, Indianapolis, IN.*

Background/Purpose: Baricitinib was evaluated for carcinogenicity in a 2 year Sprague-Dawley rat study and a 6 month HRAS transgenic mouse study to support clinical development for rheumatoid arthritis. Methods: Baricitinib was administered daily by oral gavage to HRAS mice for 26 weeks at dose levels of 0, 15, 40, or 300 mg/kg for males or 0, 10, 30, or 150 mg/kg for females. Baricitinib was administered to male and female rats daily by oral gavage at dose levels of 0, 1, 3 or 8 mg/kg for the males and 0, 3, 8 or 25 mg/kg for females for at least 90 weeks. Mortality, clinical observations, food consumption, toxicokinetics and clinical and anatomic pathology were assessed. Results: In mice, no effects on survival and no microscopic evidence of increased carcinogenicity were observed. Nonneoplastic findings consisted of bone marrow hypocellularity and increased adipocytes. The no observed effect level (NOEL) for neoplasia and the no observed adverse effect level (NOAEL) for nonneoplastic findings for baricitinib in HRAS mice is 150 mg/kg for females and 300 mg/kg for males. The AUC exposures at the NOEL in males and females are approximately 47- and 66-fold greater than AUC exposures after administration of the expected maximum human dose of 4 mg/day for rheumatoid arthritis, respectively. In rats, baricitinib was well tolerated and generally was associated with a dose dependent increase in survival. Baricitinib-related increased survival was attributed to the decreased incidence of hematopoietic neoplasms and mammary neoplasms, likely secondary to test article-related decreased weight gain. There were also decreases in the incidence of proliferative changes including decreased incidences of neoplastic and hyperplastic lesions in the mammary gland of females, and in the liver of males and females. No neoplasms were associated with the administration of baricitinib at any dose level. Therefore baricitinib was not considered to be carcinogenic at dose levels up to 8 mg/kg in males and 25 mg/kg in females. The margins of safety in males and females to the proposed maximum clinical dose of 4 mg/day for rheumatoid arthritis are approximately 6- and 26-fold, respectively. Conclusion: Administration of baricitinib was well tolerated in both rat and mouse carcinogenicity studies and did not produce neoplasms at any of the administered doses up to 66X the maximum projected human dose. In rats, baricitinib administration was associated with increased survival and a decrease in proliferative changes.

1649 Comparison of Selected Clinical Pathology Parameters in Two Strains of Rats in Different Cage Environments Used in Toxicology Studies

A. Adamou and C. Copeman. *Charles River, Montreal, QC, Canada.* Sponsor: M. Vezina.

Rodents on toxicology studies have historically been housed in stainless steel cages. Changes in housing requirements in part ensuing from guidance from the European Union have resulted in more studies being run using solid bottom polycarbonate cages with bedding material. Comparative data from rodents housed in conventional metal (stainless steel wire mesh bottom or perforated floor cages) or polycarbonate (solid bottom) cages containing bedding were reviewed to assess whether environmental conditions have an effect on background clinical pathology profiles. Parameters of interest for comparison included hematology (red blood cell count, white blood cell count, platelet count) and clinical chemistry (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, glucose, cholesterol, triglycerides, total protein, albumin, globulin, albumin/globulin ratio, calcium) endpoints. Hematology endpoints were comparable for Sprague Dawley (SD) rats housed in the different cage environments in our laboratory. Mean white blood cell counts for Wistar rats were higher (39.9% and 45.8% for males and females, respectively) for those rats housed in stainless steel cages; however, the values were well within the historical reference ranges for this strain at our facility. Clinical chemistry endpoints, with the exception of triglycerides, were comparable for both Sprague-Dawley and Wistar rats housed in the different cage environments. Range of triglyceride levels for male and female Wistar rats housed in polycarbonate bins were higher (162% and 325% for males and females, respectively) when compared to those of rats housed in stainless steel perforated floor cages and were outside the higher end of the historical reference ranges at our facility. In conclusion, based on the data collected, changing the housing conditions for SD and Wistar rats would have no apparent impact on the objectives or conclusions of a toxicology study.

1650 Nonclinical Safety Profile of Ponatinib, a Tyrosine Kinase Inhibitor for the Treatment of Chronic Myeloid Leukemia

J. Singh, J. Iuliucci and N. Narasimhan. *Preclinical Safety, Ariad Pharmaceuticals, Cambridge, MA.*

Ponatinib is an oral tyrosine kinase inhibitor (TKI) with potent activity against native BCR-ABL and resistant mutants, including T315I. Here we describe nonclinical safety studies conducted per ICHS9 guidelines. *In vitro* studies included mutagenicity and hERG ion channel assays. *In vivo* oral studies included micronucleus study in mice; ≤6 month toxicity, phototoxicity, developmental, and juvenile toxicity studies in rats, and ≤6 month toxicity studies in monkeys. Single dose pulmonary, neurofunctional, renal, and gastrointestinal safety pharmacology studies were conducted in rodents, and cardiovascular effects were assessed in

a single-dose study in telemetered dogs. Results from our *in vitro* studies indicate that ponatinib inhibited the hERG channel with an IC50 value 16x the total Cmax at the human therapeutic dose. Ponatinib was not mutagenic. In the rat phototoxicity study, ponatinib did not cause any dermal changes, but caused corneal edema. Mild renal and gastrointestinal effects were noted in safety pharmacology studies in rats. There were no cardiovascular effects in dogs. Toxicities were generally consistent between rats and monkeys; rats were more sensitive. Key effects included skin erythema, decreased T3 and increased T4 and TSH levels with thyroid follicular atrophy, diarrhea with gastrointestinal necrosis, increased hepatic transaminases with no morphological correlate, increased BUN and creatinine with renal tubular necrosis, increased lipase with pancreatic acinar cell necrosis, degeneration of testicular germ cell epithelium, and ovarian follicular atresia. Systemic exposure was dose-related in both species; exposure multiples at tolerated doses were sub-therapeutic. There were no effects on developmental parameters assessed in juvenile rats. Embryofetal toxicity was observed in rats. Skin, liver, kidney, thyroid, pancreatic, gastrointestinal, and hematopoietic toxicities were noted in humans, as consistent with animal studies. Overall, the nonclinical safety profile supported registration and informed clinical safety monitoring.

PS 1652 A 4-Week Repeated-Dose Toxicity Study of TPN902 in Beagle Dogs

L. Gong, J. Yao, J. Wang, R. Tan, G. Huang, J. Li, Z. Wang, J. Shen, H. Jiang and J. Ren. *Center for Drug Safety Evaluation and Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China; Department of Medicinal chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China; Drug Discovery and Design Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China.*

TPN902 is a multi-targeted drug candidate with a novel chemical structure for treating schizophrenia acting as a regulator for dopamine D2 receptor, 5-HT2A receptor and 5-HT1A receptor. TPN902 was effective in treating phencyclidine-induced psychosis at doses as low as 0.03mg/kg in mice. The objective of this study was to evaluate the potential toxicity of TPN902 in Beagle dogs for its submission as an investigational new drug. TPN902 was orally administered to dogs (5/sex/dose) at doses of 0 (0.5% CMC-Na, vehicle control), 0.04, 0.2 and 1.0 mg/kg once daily for 4 weeks followed by a 4-week recovery period. Toxicity was assessed by examining clinical signs, body weight, food consumption, body temperature, ophthalmoscopy, ECG, blood chemistry, hematology, coagulation, urinalysis, gross pathology, organ weights and histopathology. Key findings were as follows. Tremors and decreased motor activity were observed at 0.2 and 1.0 mg/kg, with higher frequency and severity occurred at 1.0 mg/kg. These effects were likely an exaggerated pharmacological effect of TPN902. Decreased body weight and food consumption were noted at 1.0 mg/kg/day in both sexes during the first week of the dosing phase and recovered gradually afterwards. The serum prolactin levels were increased in both sexes on days 1 and 28 after treatment with a dose dependant manner showed on day 28. Lobular hyperplasia and milk secretion in the mammary tissue, hypertrophy of corpora lutea in the ovary were present in female animals at all dose levels, which were considered to be pharmacologically mediated hyperprolactinemia as a consequence of D2 antagonistic activity of TPN902. These changes were recovered after the 4-week recovery period. Taken together, we concluded that a dose of 0.04 mg/kg/day was the no-observed-adverse-effect level (NOAEL) in both sexes. At the NOAEL, the Cmax and AUC0-24h of TPN902 at week 4 were 21.4±14.5 ng/mL and 316.0±263.2 ng•h/mL, respectively, for males and 15.7±7.8 ng/mL and 211.0±131.3 ng•h/mL, respectively, for females.

PS 1653 Chaperone-mediated Autophagy in Renal Tubules After Treatment with a PEG-Linked Protein: *In Vitro* and *In Vivo* Tools for Early Screening of Reversible Kidney Tubular Epithelial Cell Toxicity

J. E. McDuffie, S. Lee, J. Y. Ma, J. Kanerva, Y. Chen, S. Snook and F. Schoetens. *Preclinical Development & Safety, Janssen Research & Development, LLC, San Diego, CA.*

When repeatedly given at appropriate high doses, unconjugated high molecular weight polyethylene glycols (HMW PEGs) and HMW PEG-linked proteins with molecular weights <70 kDa may induce cytoplasmic renal cortical tubular epithelial cell vacuolation (RTECV) in research animal models as an adaptive response following acute and chronic compound administration. The mechanisms responsible for this phenomenon remain unclear. Regulatory authorities continue to encourage pharma to conduct exploratory studies to evaluate the translational relevance of treatment related kidney toxicity and associated changes in drug-induced kidney injury (DIKI) biomarkers. Therefore, we sought to identify biomarkers to enable monitoring for drug-induced RTECV in adult male Sprague Dawley (SD) rats, adult rat renal tubular epithelial cell (rRTEC) cultures and adult human renal tubular epithelial cell (huRTEC) cultures. Animals and cells were exposed to a proprietary 30 kDa PEGylated Peptide candidate therapeutic. Where applicable, the time course of progressive and reversible RTECV and associated changes in traditional biochemistry parameters, kidney histopathology findings identified using routine light microscopy and electron microscopy, immunopositive staining in kidney sections, gene and protein expression profiles identified using reverse transcriptase quantitative-polymerase chain reaction (RTq-PCR), western blot and immunoassays were determined in representative kidneys, serum, urine, cell lysates and/or culture medium. Taken together, 30 kDa PEGylated Peptide-induced RTECV appeared as an adaptive response characterized by biomarker profile changes indicative of reversible chaperone-mediated micro and macro autophagy in male SD rats, rRTEC and huRTEC cultures.

PS 1654 Development of a PD-1 Receptor Occupation Method in Cynomolgus Whole Blood

S. A. Kirk, A. Head and K. Troth. *Safety Assessment, Covance Laboratories Ltd, Harrogate, United Kingdom.*

Recent years have seen an increase in "checkpoint inhibitors" as potential therapies for a variety of malignancies. One checkpoint inhibitor of particular interest is the PD-1 cell surface protein expressed on activated T cells. Binding of PD-1 by its ligand PD-L1 has been shown to induce apoptosis in activated T cells and this mechanism has been exploited by certain malignancies evading immune surveillance. Therefore blocking the PD-1 and PD-L1 interaction offers a potential anti-cancer therapy. During the pre-clinical development of anti-PD1 antibody therapies, it is desirable to be able to measure exposure to the antibody by measuring the levels in the serum as well as the amount bound to the surface of PD-1 expressing cells. In this study we have developed a method to measure receptor occupancy in the monkey using a commercially available anti-PD1 antibody. Using fresh primate whole blood (collected in NaHep), two separate assays were developed. The first was to determine the proportion of bound/total receptors, and the second to determine the level of free receptors. Determination of bound/total receptors was evaluated by incubating whole blood with anti-PD-1, then by adding a labelled detection antibody (anti-mouse Ig-APC). Free receptors were determined by direct labelling of whole blood with a labelled anti-PD-1 antibody. Development of both assays required titration of primary and secondary antibodies in order to establish optimum staining conditions, typically just below the end-point of the titration curve. Results of initial runs have satisfactory titration curves for both the bound/total and the free receptor assays. Bound/total assay has shown that 100% occupation of PD-1 receptors was established between 25 and 50µg/mL and that titration of anti-PD-1 established that complete blocking of all receptors occurred between 50 and 100 µg/mL. Further work will examine the effect of full anti-PD1 receptor occupation in PBMCs on cytokine release following *in vitro* stimulation In conclusion, we have developed a method for measuring anti-PD-1 receptor occupancy with a view to adapting the procedure to support pre-clinical development of novel anti-PD-1 therapies.

PS 1655 Comparative Toxicokinetics and Toxicity of ST-101 (Olmesartan Medoxomil/Rosuvastatin Calcium) versus Single Agents in Beagle Dogs

O. J. D'Cruz[1,2], M. Munsif[1] and L. Hwang[1]. [1]*Autotelic Inc, Fountain Valley, CA and* [2]*Stocosil Inc, City of Industry, CA.*

Study Purpose: Hypertension and hyperlipidemia are independent risk factors for the development of premature cardiovascular disease (CVD). ST-101 is a fixed-dose combination of olmesartan medoxomil (OM), an angiotensin II receptor blocker and rosuvastatin calcium (RC), a hydroxymethylglutaryl-coenzyme A reductase inhibitor, is the first combination designed to treat these two risk factors. Here we compare the toxicity and toxicokinetics of OM/RC combination versus OM/RC alone. Methods: Toxicity and toxicokinetics for single agents were obtained from published literature and SBA of Benicar and Crestor. The data were supplemented with internal study conducted in 72 Beagle dogs. Dose range of OM was 125, 160, 250, and 500 mg/kg/day; Dose range of RC was 7.5, 10, 15, and 30 mg/kg/day; Dose range of OM/RC was 80/5. 120/7.5, and 160/10 mg/kg/day. Results: For OM, the key findings were: Blood urea nitrogen (BUN) and creatinine (Cr) were elevated moderately (<2-fold) and mild dilatation of renal tubules with mild to moderate regeneration of tubular epithelium was observed at doses as low as 125 mg/kg/day (only in 1 of 6 animals at this dose); at 500 mg/kg/day 1 of

6 animals had to be sacrificed in moribund condition (in renal failure) after about 1 month of treatment. There was an absence of dose-dependency and gender differences for AUC and Cmax values. For RC, the key histopathological findings were observed in gall bladder (hemorrhage and inflammatory cell infiltration in lamina propria mucosa) at all doses, and lens at 30 mg/kg. Gallbladder effects have also been observed with other drugs of this class. No apparent renal toxicity was noted. Dose dependent increase in drug exposure that is independent of sex was observed. For OM/RC combination, the main findings were renal tubule regeneration and renal tubule dilation in the high dose groups (160 mg/kg [6/12] and OM 160/RC 10 mg/kg. The reversible kidney toxicity was considered as related with olmesartan. There was no drug-drug interaction in PK - same PKs were observed for combination agent versus single agents. Conclusions: Oral administration of OM and RC combinations to Beagle dogs for 3-months indicated no pharmacokinetic drug-drug interactions between the active components of ST-101 in toxicology and toxicokinetics.

1656 Mitochondria-Targeted Platinum Coordination Compounds May Increase Cell Adhesion and Inhibit Cell Migration

L. Zhang[5], J. Li[5], X. He[1], X. Lv[3], D. Chen[5], L. Li[1], C. Chen[2], Y. Zhao[2], F. Wang[3], N. Monteiro-Riviere[4] and Z. Guo[5]. *[1]Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; [2]Institute of High Energy Physics, Chinese Academy of Sciences, Beijing, China; [3]Shanghai University of Chinese Traditional Medicine, Shanghai, China; [4]Nanotechnology Innovation Center of Kansas State (NICKS), Department of Anatomy and Physiology, Coll, Kansas State University, Manhattan, KS and [5]School for Radiological and interdisciplinary Sciences, Soochow University, Suzhou, China.*

A large number of platinum coordination compounds have been investigated for their tumor therapy ability by exerting their DNA-damage activity leading to cell death. In this work, a novel platinum compound targeted to mitochondria, namely PIP, was synthesized. Transmission electron microscopy showed the disruption of mitochondria outer membrane and cristae. Decreased mitochondria membrane potential, cytochrome C translocation, increased reactive oxygen species, decreased ATP, cell apoptosis and increased Bax/Bcl2 ratio were found, indicating PIP induced mitochondria injury. In addition, PIP exhibited high cell adhesion ability compared to cisplatin, a traditional platinum compound. PIP can inhibit cell migration and invasion, proved by wound healing assay, transwell assay, and spheroid expansion assay. Focal adhesion kinase can be phosphorylated and activated by PIP within 30min, indicating fast response of FAK and adhesion by PIP. Adhesion related molecule β-catenin translocated to the cell membrane when cells were treated with PIP. The mechanistic studies showed that Wnt signal pathway and nuclei translocation of β-catenin can be blocked by PIP. Accordingly, the above findings elucidated the mitochondria targeting and migration inhibition properties of PIP with associated molecular mechanisms, providing a novel method for cancer therapy.

1657 Age-Related Acute Thrombocytopenia in Cynomolgus Monkeys Induced by a Therapeutic Monoclonal Antibody

J. Sun[2], Y. Zhang[2], H. Jiang[1], Z. Li[1], L. Gong[2] and J. Ren[2]. *[1]Shanghai Jiaotong University School of Medicine, Shanghai Cancer Institute, Shanghai, China and [2]Center for Drug Safety Evaluation and Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China.*

Single intravenous administration with CH12 (a therapeutic monoclonal antibody) at ≥25mg/kg induced acute thrombocytopenia (4 hours post dosing) in 3-4 years old, but significantly weak in 6-7 years old Cynomolgus monkeys, but not in rats *in vivo*. The age-related acute thrombocytopenia in monkeys was then investigated in this study. *In vitro*, after incubation of CH12 with washed platelets (wPLT) or whole blood (WB), dose-related binding and activation (CD62P expression) were found in cynomolgus platelets. Moreover, CH12 induced platelet activation was directly correlated with its binding. The binding activity of CH12 was significantly lower in samples from 6-7 years old monkeys than those from 3-4 years old monkeys. The distributions of platelet membrane proteins from 3-4 years old Cynomolgus monkeys were further compared with those from 6-7 years old using proteome method. The results showed that changes of several proteins on the platelet membrane of monkeys may contribute to the age-related acute thrombocytopenia. Additional studies are needed to find out the key platelet membrane protein of Cynomolgus monkeys contributing to the cynomolgus -specific thrombocytopenia caused by CH12 and to clarify the related molecular mechanism.

1658 Antisense Oligonucleotide Gapmers Demonstrate Heparin-Induced Thrombocytopenia Potential in a Selective Manner: Proposal of a Novel Mechanism of Toxicity

D. Petrucci[1], D. Exler[1], A. Murray[1], S. Kaushik[1], T. Knox[1], S. Fenner[4], B. Andrews[4], J. Parry[3], K. Frazier[1] and K. French[1]. *[1]Integrated Biological Sciences, GlaxoSmithKline, King of Prussia, PA; [2]Mechanistic Safety and Disposition, GlaxoSmithKline, King of Prussia, PA; [3]Mechanistic Safety and Disposition, GlaxoSmithKline, Ware, United Kingdom and [4]Product Development, GlaxoSmithKline, Stevenage, United Kingdom.*

Thrombocytopenia is a common toxicity observed both clinically and preclinically with antisense oligonucleotides (ASO). Recently, a potential mechanism was identified, where nucleic acids and aptamers were shown to alter the antigenic properties of the small cytokine platelet factor 4 (PF4) in a similar manner as observed in heparin-induced thrombocytopenia (HIT). These effects were shown to be proportional to nucleotide length (>20mer active) and/or secondary structure. Therefore, it was believed that ASOs of therapeutically relevant lengths (≤ 20mer) would not demonstrate HIT potential. We evaluated HIT potential of 10 ASOs using an ELISA based approach. The panel consisted of 5 gapmers with locked nucleic acid (LNA) modifications and 5 gapmers with 2′ O-Methyl (2OMe) modifications, which ranged from 14 to 20 nucleotides in length. In the ELISA assay, half of the gapmers were considered positive as shown by a similar or greater fold increase in gapmer/PF4/anti-PF4-heparin complex antibody (KKO clone) binding versus the positive control heparin. The positive gapmer range of fold changes was from 5- to 19-fold binding over absence of PF4 versus the heparin fold change of 5, and all occurred in a bell shaped manner. The gapmer Cmax range (0.1 to 100 mcg/mL) was within clinical or preclinical exposures with other ASOs. Both longer gapmers and 2OMe gapmers tended to have higher binding (4 of 5 20mers and 4 of 5 2OMe gapmers). The mechanism of the trend in 2OMe-related activity is unclear, but may be related to a steric-dependent effect. In conclusion, we have identified a novel potential mechanism for the ASO class effect of thrombocytopenia. Additional gapmers will need to be tested in order to gain further understanding of the structure activity relationship of base modifications and base length on HIT potential.

1659 Safety Profile of BT-11: A Novel LANCL2-Based Therapeutic for Crohn's Disease

M. Ehrich[2], P. Bissel[2], K. Boes[2], J. Hinckley[2], B. S. Jortner[2], G. Magnin-Bissel[2], S. R. Werre[2], A. Carbo[1], R. Montecillas[1], C. Philipson[1], R. D. Gandour[1] and J. Bassaganya-Riera[1]. *[1]Biotherapeutics, Inc., Blacksburg, VA and [2]Virginia-Maryland College of Veterinary M, Blacksburg, VA.*

Inflammatory Bowel Disease (IBD) is a chronic, recurring disease of the gastrointestinal tract that afflicts over 4M people worldwide. Current therapies for Crohn's disease (CD) are modestly successful and associated with significant adverse side effects. BioTherapeutics validated Lanthionine Synthetase Cyclase-Like 2 (LANCL2) as a novel therapeutic target for CD and developed a small molecule clinical lead, BT-11, that binds to LANCL2, is orally active, acts locally in the gut, and has demonstrated therapeutic efficacy in 3 validated mouse models of colitis at doses of 8 mg/kg/da. To evaluate BT-11's safety and pharmacokinetic (PK) profiles, adult Harlan Sprague Dawley rats were given vehicle, a single 500 mg/kg oral dose or repeated oral doses of 80 mg/kg/da for 14 days, with behavior assessed and blood and tissues collected at 1-4, 7-8, 14 and 21 days. Results indicated that BT-11 is safe and well tolerated across doses. Functional observational battery (FOB) endpoints demonstrated no consistent differences between treated and control groups over multiple times of observation for quantal (normal/abnormal) endpoints (e.g., oculocardiac reflex, visual placing), endpoints in numerical categories (e.g., posture, response to touch), or measured endpoints (e.g., weight, grip strength, body temperature, time on a rotarod). Histopathological examination of brain, kidney, liver, adrenal gland, testes, stomach, small and large intestines, duodenum, pancreas, heart, lungs, spleen, thymus, rib and leg bone found no damage among the groups at any of the post-dosing intervals. Liver enzymes were elevated 2-4 days after the 500 mg/kg single dose but returned to normal values by 8 days and were not observed at any time in rats

given 80 mg/kg/da for 14 days. These results demonstrate an ample therapeutic window and minimal toxicological significance of the high-dose finding. PK analyses revealed a high oral apparent volume of distribution, suggesting local effects at gut mucosa. In conclusion, oral administration of BT-11 is safe for rats and a promising safe and effective CD therapeutic. Supported by the Virginia Biosciences Health Research Corporation (VBHRC) and federal SBIR/STTR funds (1R43DK097940 and 1R41DK099027).

1660 Seizure Risk Assessment Using Rat Telemetry Electroencepharography (EEG) System; Pentylenetetrazole and 4-Aminopyridine Induced Different Effects on EEG

K. Watanabe, T. Tsubouchi, K. Ikeda and H. Funabashi. *Preclinical Research Laboratories, Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan.* Sponsor: T. Yamada.

Introduction: Since seizure observed in preclinical studies has a great influence on the development of new drugs, the seizure risk should be carefully assessed and managed before clinical trials. Since some abnormal EEG changes can be noted before the occurrence of drug-induced seizure in both animals and humans, it has been recognized that the EEG changes can be premonitory signs for the seizure. In the present study, we investigated the effects of 2 proconvulsants, pentylenetetrazole (PTZ) and 4-aminopyridine (4-AP), on the EEG signals in conscious rats. Method: Male Sprague-Dawley rats were used. EEG electrodes were implanted into the cranial bone (positive lead; AP-2.0 mm ML+3.0 mm, negative lead; AP-6.0 mm ML-3.0 mm). PTZ and 4-AP were intraperitoneally administered at 10 to 60 mg/kg and 1 to 7 mg/kg, respectively. Continuous telemetry EEG measurement and clinical observation were simultaneously performed from before administration until 2 hours after administration. Results: PTZ caused clonic convulsion at 45 and 60 mg/kg accompanied with typical EEG waveforms (convulsion EEG). At lower doses over 30 mg/kg of PTZ, all rats showed rhythmic spikes and twitch. Although 4-AP also caused clonic convulsion accompanied with the convulsion EEG at 3 to 7 mg/kg, the rhythmic spikes were never noted at any dose levels. Tremor and twitch were noted at lower doses over 2 mg/kg. Discussion: In PTZ and 4-AP, twitch and/or tremor were noted at lower dose than that caused convulsion. Therefore, the signs can be useful as the premonitory sign for seizure. Whereas rhythmic spikes were noted after PTZ administration at lower dose than that caused convulsion, the spikes were never noted after 4-AP administration. Based on the results, the rhythmic spikes are considered to be a good premonitory sign for drug-induced seizure, especially GABAA inhibition-induced seizure but not for K+ channel inhibition-induced seizure. Detailed neurobehavioral observation and considering of the seizure mechanism will also help the seizure liability. We further study the different effects of PTZ and 4-AP on the EEG signals.

1661 Anxiolytic Effects of Noribogaine on Novelty Stress in Rats and Zebrafish

X. Li[2], A. V. Kalueff[3] and E. L. Maillet[1]. [1]*DemeRx, Inc, Miami, FL;* [2]*ITR Laboratories Canada Inc, Baie d'Urfe, QC, Canada and* [3]*ZENEREI LLC, Slidell, LA.*

Recent molecular evidence suggests that noribogaine is relevant to novel therapeutic approaches to treat addiction and other brain ailments. Here, we report the effects of noribogaine in established paradigms relevant to novelty stress and anxiety in two animal models. Adult female Sprague-Dawley rats were subjected to Functional Observational Battery testing following a single oral dose of noribogaine (25, 50, 100 mg/kg) or vehicle. Each group (10 per group) was assessed at predose, 1, 2, 6 and 24 hours post-dose. Noribogaine treated rats displayed transient and dose-dependent reductions in the frequency of rearing, exploratory activity and arousal between 1 and 6 hours post-dose (peaked at 2 hours) in the open field test; no changes were noted in activity, alertness, posture, sensorimotor responsiveness, neuromuscular or autonomic effects. Adult zebrafish (*Danio rerio*, 15 per group) were subjected to the novel tank test following drug immersion treatment (1, 5 and 10 mg/L noribogaine) or water only. Each group was observed for 5 minutes. Noribogaine produced robust anxiolytic-like behavior in fish as revealed by the prolonged time spent in the top half compartment, increased number of transitions to top, and the absence of freeze-bouts (associated with fear and anxiety) in control fish. Taken together, these data obtained from rats and zebra fish indicate noribogaine modulates the components of the acute stress response, usually associated to emotionality and anxiety in these paradigms. The consistent observations in these two relatively distant classes of vertebrate show the mechanism of action for noribogaine is most likely related to the effects targeted to a highly conserved neurological structure(s) such as hypothalamic-pituitary-adrenal axis.

1662 Effects of *Argemone mexicana* Methanolic Leaf Extract on Antioxidant and Liver Phase-One Oxidizing Enzymes During Malaria Infection in Mice

E. A. Balogun[3], T. O. Sunmonu[1], A. I. Adeogun[2] and T. Popoola[2]. [1]*Biochemistry, Ahlikman University, Ilorin, Nigeria;* [2]*Biochemistry, University of Agric., Abeokuta, Nigeria and* [3]*Biochemistry, University of Ilorin, Ilorin, Nigeria.*

The search for new antimalarial drugs has become necessary due to the resistance of malaria parasites to existing drugs. *Argemone mexicana* Linn. has been used against various diseases including malaria. The antimalarial activity of the methanolic leaf extract of *Argemone mexicana* at different dosages and its effects on some biochemical parameters were investigated in *Plasmodium berghei* infected mice. Parasitaemia, body weight, packed cell volume (PCV), aniline hydroxylase (A4H), aminopyrine N-demethylase (ANDH), catalase (CAT), uperoxide dismutase (SOD) glutathione peroxidase (GPx) and glutathione transferase (GST) were investigated. Mice were infected by intraperitoneal injection of standard inoculum of chloroquine-sensitive *Plasmodium bergei* (NK65). The animals were divided into six groups of twelve mice each. Gp 1 normal control, Gp 2 infected untreated,Gps 3–5 were infected and orally administered 100, 200, 300mg/kgbwt of extract. Gp 6 was infected and administered 10mg/kgbwt chloroquine (CQ) the reference drug. The infected untreated animals had a significant increase ($p<0.05$) in parasitaemia and malondialdehyde (MDA) concentration(53%) while the body weight and PCV level were reduced significantly. The activities of CAT, SOD, GPx and GST were significantly reduced ($p<0.05$) by 51%, 49%, 33%, and 47% in the compared with the control, A4H, and ANDH, also showed significant reduction ($p< 0.05$) in activity by 43% and 61%. The extract significantly suppressed parasitaemia and enhanced body weight as well as PCV in a dose dependent manner. At a dose of 300mg/kgbw, it suppressed parasitaemia by 97% while CQ by 100%.The leaf extract restored the activities of the antioxidants and liver phase - one enzymes compared with the control thereby attenuating MDA level. *A. mexicana* at the dose of 300 mg/kgbwt of the extract compared favorably with chloroquine, a standard antimalarial drug which confirmed the antimalarial activity of the leaf extract.

1663 Synthesis of Potent and Selective AKR1C3 Inhibitors for Acute Myeloid Leukemia Therapy

K. Verma[1], T. Zhang[2], T. M. Penning[2] and P. C. Trippier[1]. [1]*Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Amarillo, TX and* [2]*Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, PA.*

Background: Human aldo-keto reductase 1C3 (AKR1C3) enzyme belongs to the aldo-keto reductase superfamily of enzymes, a group of oxidoreductases which catalyze the NADP(H) dependent reduction of aldehyde and ketone functionalities. The enzyme is upregulated in Acute Myeloid Leukemia (AML) where it functions to catalyze the conversion of prostaglandin (PG) PGD2 to 11β-PGF2α. Formation of 11β-PGF2α deprives the peroxisome proliferator-activated receptor γ of its ligands and brings about the proliferation of AML cells. Inhibition of the 1C3 isoform diverts prostanoid synthesis to the production of PGJ2, responsible for differentiating AML cells. The enzyme shares close homology to its isoforms AKR1C1 and AKR1C2, which have different functions. Hence, the synthesis of potent and selective AKR1C3 inhibitors is paramount. While inhibitors of AKR1C3 have been identified with nanomolar potency and selectivity, until they reach the clinic there is a need for additional lead compounds. The structurally novel natural product baccharin, isolated from Brazilian green propolis exhibits potent AKR1C3 inhibition activity (IC50 =100 nM) and crucially, exquisite selectivity for the AKR1C3 isoform. However, the presence of an ester side chain makes the molecule labile to hydrolysis by cellular esterases to yield an inactive metabolite drupanin. Methods: We have identified a preliminary structure-activity relationship of the baccharin scaffold and designed metabolically stable compounds that exert up to two fold enhanced potency than baccharin. These analogues were assayed for their cytotoxic effect alone and in combination with etoposide (a clinical chemotherapeutic) in HL-60 AML cells. Results and Conclusions: Baccharin analogues when delivered with etoposide demonstrate a significant potentiation effect, enhancing the cytotoxicity of etoposide by up to six fold. Etoposide (1 μM) provides approximately 34 % reduction of cell viability in a human AML cellular model. Application of 1 μM etoposide with 0.1 μM of our non-toxic lead compound resulted in significant synergism between the two agents since this resulted in complete abrogation of leukemia cell viability: an adjuvant effect far greater than reported by pan-AKR1C inhibitors.

1664 Blockade of IL-1 Signaling Overcomes Resistance to Erlotinib in Head and Neck Squamous Cell Carcinoma

A. Stanam, L. Love-Homan, M. Espinosa-Cotton and A. Simons-Burnett. *Pathology, University of Iowa, Iowa City, IA.*

Tumor resistance to epidermal growth factor inhibitors (EGFRIs) such as erlotinib and cetuximab is a significant impediment in the success of targeted therapy in head and neck squamous cell carcinoma (HNSCC). Therefore, the investigation of novel strategies to overcome EGFRI resistance is imperative to improve the survival odds of HNSCC patients. Prior work in our lab showed interleukin-1 alpha (IL1α) signaling was involved in the reduced anti-tumor efficacy of EGFRIs in HNSCC cells suggesting that IL-1 signaling was important for tumor response to EGFRIs. Therefore this study's objective is to determine if IL-1 pathway blockade would overcome resistance to erlotinib in HNSCC *in vitro* and *in vivo*. Augmented levels of IL1A and interleukin-1 receptor accessory protein (IL1RAP) mRNA were independently associated with worsened patients' survival as per 'The Cancer Genome Atlas' analysis of HNSCC gene expression dataset. Erlotinib-resistant (ER) cell lines were developed from erlotinib-sensitive (ES) HNSCC cell lines by culturing them in the continuous presence of erlotinib for 3-4 months. Gene enrichment analysis of microarray data revealed a significant upregulation of genes involved in IL-1 signaling pathway in ER-SQ20B and -Cal27 cell lines compared to their respective ES counterparts. Gene expression (validated by RT-PCR) of IL-1 ligands i.e. IL1A and IL1B were significantly upregulated by > 2 fold in both the ER cell lines versus ES cell lines. Additionally, there was an up regulation of genes involved in cytosolic signaling of IL-1 i.e. TOLLIP = 1.7 fold, TRAF6 = 2.9 fold, IRAK1 = 1.5 fold and canonical IL-1 target genes i.e. PTGS2 = 4.4 fold, DUSP1 = 1.5 fold in ER-SQ20B cells versus their ES-cells. Blockade of IL-1 signaling using the recombinant IL-1R antagonist (r IL-1Ra/anakinra) was able to overcome resistance to erlotinib in SQ20B and Cal-27 tumors *in vivo* but not *in vitro*. The effect of IL-1 blockade on tumor microenvironment is currently being investigated. Altogether, IL-1 pathway blockade overcame erlotinib resistance in HNSCC cells and may represent a novel strategy to overcome EGFRI resistance for treatment of HNSCC patients.

1665 An Early Screening Model in the Conscious Dog with Simultaneous Evaluation of Cardiovascular and Respiratory Functions

S. Rompion. *Porsolt Sas, Le Genest Saint Isle, France.* Sponsor: G. Froget.

In safety pharmacology, the effects of new chemical entities on respiratory and cardiovascular function are usually evaluated in rodents using plethysmography and in large animals using telemetry, respectively. Simultaneous monitoring of respiratory and cardiovascular parameters in the same large conscious animals could be advantageous from scientific and ethical points of view. The purpose of the present study was to evaluate the performances of the Jacketed External Telemetry (JET) system for accurate measurement of ventilatory parameters in the conscious non-restrained telemetered dog. A pharmacological validation was performed using theophylline, a respiratory stimulant reference drug usually used in respiratory studies. Male and female Beagle dogs (n=5) implanted with DSI telemetric devices wore a jacket with external telemetry equipment and respiratory inductive plethysmography bands for at least 24 hours. The respiratory (respiratory rate, RR and minute volume, MV) and cardiovascular (blood pressure, MBP and heart rate, HR) parameters were recorded in two successive sessions at 98-hour interval. The effects of theophylline (40 mg/kg, p.o.) were investigated in the same conscious non-restrained dog. After a habituation period, the cardio-respiratory parameters were monitored prior to and up to 16 hours post-dosing. Data with theophylline were compared with a vehicle control and were analyzed by two-way ANOVA (group, time). Pre-dose values recorded in all animals on two separate occasions demonstrated the stability of respiratory parameters in telemetered dogs. Theophylline (40 mg/kg, p.o.) exhibited respiratory stimulant properties, as compared with vehicle controls (MV, +254%; RR, +248%, $p<0.05$ for each) with concomitant tachycardia (HR, +86%, $p<0.01$) and a trend towards an increase in MBP (+20%, NS). The JET system is a non-invasive technique allowing for the measurement of ventilatory parameters in freely moving animals. Although this approach does not allow precise assessment of pulmonary mechanics, it is able to evaluate the main effects of drug candidates on cardio-respiratory functions. The simultaneous monitoring of respiratory and cardiovascular parameters can therefore be included as an early screening model in programs of safety pharmacology.

1666 PAK1/2 Kinase Inhibitors Cause Acute On-Target Cardiovascular Toxicity in Rodents

Y. Zhong, G. Cain, P. Katavolos, R. Vega, C. Chou, H. La, L. Murray, T. O'Brien, C. Ndubaku, J. Rudolph and D. Diaz. *Genentech, South San Francisco, CA.*

p21-Activated kinases (PAKs) are STE20 family Ser/Thr kinases. Based on sequence homology, PAKs are subdivided into two groups: Group I (PAK1, 2, and 3) and Group II (PAK4, 5, and 6). PAK1 modulates the activity of down-steam kinases important in cellular proliferation, survival and motility. Thus PAK1 inhibitors may represent a novel and rational targeted therapy for human malignancies. Due to the high sequence homology of Group I PAKs, our small molecule PAK1 inhibitors also inhibit PAK2 and PAK3. PAK3 is mainly expressed in the brain and our compounds have little brain penetration thus do not inhibit PAK3 *in vivo*. We found that selective PAK1/2 inhibitors caused acute (1-4h after dosing) cardiovascular toxicity and intolerability in rats and mice with therapeutic index <1. Literature evidence on KO phenotype and target expression was not sufficient to allow us to identify an underlying mechanism. We conducted thorough mechanistic investigations including the use of chemically diverse tool molecules and inactive analogs of our PAK1/2 inhibitors. We found that molecules with no overlapping off-targets caused similar cardiovascular toxicity in mice. Across 3 chemotypes there was a strong correlation between the unbound Cmax that caused lethality *in vivo* and the cellular potency on PAK1. Our data strongly support a weight-of-evidence argument that the cardiovascular toxicity by PAK1/2 inhibitors is on-target. Lessons learnt from this program resulted in the general recommendation about the usefulness of generating relevant KO or transgenic mice to inform target tractability at earlier stages for future programs.

1667 Lysosomal Adaptation: How Cells Respond to Lysosomotropic Compounds

S. Lu, T. Sung, R. Abraham and B. Jessen. *Pfizer Inc, San Diego, CA.*

Lysosomes are membrane-enclosed compartments filled with acid hydrolytic enzymes that digest macromolecules and organelles from both internal and external sources. Intracellular trafficking and degradation pathways, including endocytosis, phagocytosis and autophagy converge at the level of lysosomes, which have recently demonstrated signaling-hub characteristics. Basic lipophilic compounds have been known to accumulate in acidic lysosomes via pH partitioning (i.e. lysosomotropism) and this sequestration is believed to disturb lysosomal function. In the current study, selected lysosomotropic compounds triggered lysosomal activation demonstrated by increased lysosotracker and lysosensor staining, and increased cathepsin activity. However, the lysosomal activation was associated with lysosomal dysfunction exemplified by intracellular and extracellular substrate accumulation, including phospholipid, cholesterol, SQSTM1/p62, GAPDH and opsin. Additional studies revealed that nuclear translocation of transcriptional factors, including both TFEB and TFE3, increased with lysosomotropic compound treatment. These transcription factors could mediate the lysosomal adaptation. In addition, calcium release and reduced mTOR signaling appear to play a significant role in mediating the TFEB and TFE3 nuclear translocation. When the lysosomal adaptation is reduced, cells are more susceptible to cytotoxicity induced by lysosomotropic compounds, indicating this adaptive change benefits cells. Future work will focus on the involvement of TFEB and other transcription factors in the lysosomal change by the lysosomotropic compounds.

1668 Mechanisms for Hepatobiliary Toxicity in Rats Treated with Antagonists of Melanin Concentrating Hormone Receptor 1 (MCHR1)

M. Otieno[2], V. Bhaskaran[1], E. Janovitz[1], Y. Callejas[1], W. B. Foster[1], J. R. Megill[1], L. Lehman-McKeeman[1] and B. Gemzik[1]. [1]*Bristol-Myers Squibb, Princeton, NJ and* [2]*Janssen Pharmaceuticals (J&J), Springhouse, PA.*

Thienopyrimidone MCHR1 antagonists were being developed as a potential therapy for diabetes and obesity. However, when male SD rats and diet-induced obese rats were dosed orally for a duration of 4 to 28 days with a representative molecule(s) of this series, hepatobiliary lesions characterized by hypertrophy, hyperplasia, and individual cell necrosis of hepatocytes and bile duct hyperplasia were observed. The objective of the work presented here was to investigate mechanisms of hepatobiliary toxicity by this class of molecules and develop an *in vitro* screen to select against this liability. When rats were concurrently given the broad spectrum CYP inhibitor 1-aminobenzotriazole (ABT) with MCHR1 antagonists, there was no hepatobiliary damage suggesting a biotransformation-, and not target pharmacology-related mechanism

of toxicity. The liver gene expression profile revealed treatment-related changes in hepatic transporters and induction of oval cell-specific genes. However, *in vitro* hepatic transporter function was not altered suggesting that transporter-mediated cholestasis was not the initiating mechanism of toxicity. Dmbt1, an oval cell marker was induced *in vivo* by MCHR1 antagonists and was unchanged when biotransformation-dependent hepatobiliary injury was prevented with ABT. Dmbt1 mRNA induction and cytotoxicity was also observed in primary rat biliary epithelial cells (BECs) exposed to metabolites, but not parent molecules, which was consistent with *in vivo* results, thus rendering the *in vitro* model suitable as a screening platform. In conclusion, hepatobiliary injury by thienopyrimidinone MCHR1 antagonists was driven through an identified biotransformation pathway. Induction of Dmbt1 mRNA coupled with *in vitro* cytotoxicity in BECs was useful to monitor structural modifications to guide chemistry direction, and may provide an approach toward more broadly evaluating ability for biliary injury.

1669 Utility of 3D Human Liver Microtissues for Prediction of Compounds That Cause Drug-Induced Liver Injury in Humans

A. J. Foster[1], W. R. Proctor[2], J. Vogt[2], C. Summers[1], K. Oldman[1], M. Kijanska[3], J. Haugstetter[3], J. M. Kelm[3], D. Misner[2], P. Morgan[1], S. Messner[3] and D. P. Williams[1]. *[1]AstraZeneca, Cambrigde, United Kingdom; [2]Genentech, South San Francisco, CA and [3]InSphero AG, Schlieren, Switzerland.*

Drug-induced liver injury (DILI) is a major cause of attrition during drug development and post-market withdrawal. Early identification of DILI risk during lead optimization will result in selection of clinical candidates with more favourable safety attributes. However, current *in vitro* models of hepatotoxicity are limited to assessing acute cytotoxicity only, while the onset of DILI is often observed following several weeks of treatment. Recently, 3D InSight Human Liver Microtissues consisting of primary hepatocytes and Kupffer cells have been shown to preserve liver specific functions over several weeks in culture. This therefore enables long-term repeat dose assessments, not currently possible with 2D-hepatocyte culture systems. In this study, we assessed the predictivity of 3D Human Liver Microtissues to discriminate between DILI and non-DILI causing drugs, utilizing ATP depletion as an indicator of organoid viability. We have assessed >100 marketed drugs with known DILI potential after 5-6 or 14 days exposure time. Dose and time dependent cell toxicity was observed with compounds for which cell viability was determined after 5-6 days or 14 days exposure. Prolonged exposure resulted in decreased IC50 values for 19 out of 38 compounds. Expression of the 14 day ATP IC50 values relative to the human plasma Cmax concentration enabled determination of a "margin-of-safety" (MOS). Statistical modelling indicated that greatest sensitivity and specificity was achieved using a 30 fold MOS-threshold for distinguishing between known DILI compounds and non-DILI compounds. The 3D liver model exhibited a sensitivity of 70% for detection of known DILI causing drugs (n= 53, DILI severity category 1-3) and a specificity of 90% for correct identification of non-DILI drugs (n=38, DILI severity category 4-5). In addition, the performance of 3D Human Liver Microtissues was compared to traditional 2D-cultures of THLE-Null, THLE-3A4 cells, and primary human hepatocytes, which exhibited lower sensitivity and specificity for the prediction of DILI. We conclude that the 3D Human Liver Microtissues represents a suitable model to perform long-term *in vitro* hepatotoxicity investigations in a mechanistically relevant liver model.

1670 A High Content Imaging (HCI) BAC-GFP Toxicity Pathway Reporter Platform for Mechanism-Based Assessment of Drug-Induced Liver Injury (DILI)

B. ter Braak, S. Wink, S. Hiemstra, M. Niemeijer, S. Huppelschoten and B. van de Water. *Toxicology, Leiden University, Leiden, Netherlands.*

DILI remains a major concern for drug development and in clinical practice. At the moment primary human hepatocytes are regarded as the golden standard for DILI toxicity testing. However, problems with the availability, inter-donor variability and stability (dedifferentiation of hepatocytes and thus loss of CYP/drug metabolizing capacity) remain a critical issue. Here we present a BAC-GFP reporter platform in which we monitor the activation of mal-adaptive stress pathways that are typically activated by chemical-induced cellular injury. We have established and characterized reporters for oxidative-stress (KEAP1/Nrf2 pathway), ER-stress (UPR pathway), and DNA-damage (p53 pathway), allowing single cell time-resolved and quantitative analysis of the toxicity pathway activation. We have exposed these individual reporters to a library of >150 DILI compound and mapped the dynamic activation of these toxicity pathways in a 24 hr time period at 1, 5, 10, 50 and 100 Cmax concentrations. We applied bioinformatics tools to cluster the entire time-concentration HepG2-BAC-GFP reporter response profiles of all compounds. Our approach allows the clustering of similar mode-of-action compounds. Moreover, using our screening strategy we do enrich for compounds with severe DILI drug labeling. Since this model is still based on dedifferentiated 2D HepG2 cell lines we are actively working to integrate these reporters in human induced pluripotent stem cells (hiPSC) using the CRISPR/Cas9 system. We anticipate that our cellular stress response reporters in combination with HCI may play a key role in future safety assessment of DILI as well as other toxicity liabilities. This work was supported by the European IMI MIP-DILI and FP7 DETECTIVE projects and the Belgium IWT HILIM-3D project.

1671 Kidney Safety Biomarkers in Rodents and Non-Rodents Following Amphotericin B Administration

J. E. McDuffie[1], M. Sonee[3], J. Y. Ma[1], Y. Chen[1], J. Kanerva[1], X. Wu[2], K. Li[2], Y. Zhang[2], R. Meng[2], F. Almy[3], L. Smith[3], L. Varacallo[3], C. Lanigan[1], S. Lee[1], S. Bryant[3] , C. Johnson[3], M. Otieno[3], B. Zimmerman[3], D. Simic[3] and S. Snook[1]. *[1]Janssen Research & Development, L.L.C., San Diego, CA; [2]Janssen Research & Development, L.L.C., Shanghai, China and [3]Janssen Research & Development, L.L.C., Spring House, PA.*

From 2008–2011, the FDA, EMA and PMDA qualified 8 urinary proteins as novel drug-induced kidney injury (DIKI) biomarkers for limited use in drug development. More recently, the FDA and EMA issued letters of support for two additional novel urinary proteins. Regulatory authorities encourage pharma to conduct exploratory studies to demonstrate the translatable values for novel DIKI biomarkers. Therefore, Janssen scientists conducted investigative studies to learn about and confirm potential claims concerning the predictive values for DIKI biomarker changes in mice, rat, dogs and nonhuman primates. The nephrotoxic antifungal amphotericin B (AmpB) was administered to animals; and revealed minimal-to-moderate cortico-medullary tubular lesions in the absence of clinical signs of toxicity. Clinically relevant increases in routine serum chemistry parameters [creatinine (sCr), urea nitrogen (BUN)]; serum cystatin C (sCysC); estimated glomerular filtration rate (eGFR) and novel urinary protein DIKI biomarkers [alpha 1 microglobulin (A1M), alpha glutathione S-transferase (alpha GST), clusterin (CLU), CysC, liver-type fatty acid binding protein 1 (L-FABP1), kidney injury molecule 1 (KIM-1), N-acetyl-beta-D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), osteopontin (OPN) and total protein (TP)] were detected. Where feasible, AmpB related renal cortico-medullary tubular lesions were further characterized by evidence of significant mRNA expression level changes in macrodissected renal cortices and medullary tissues and/or immunopositive staining in damaged tubular epithelial cells. To our knowledge, this series of investigative studies provide the first illustration of the relationships between changes in kidney histopathology lesions, sCr, BUN, sCysC, eGFR, Ur A1M, Ur alpha GST, Ur CLU, Ur CysC, Ur L-FABP1, Ur KIM-1, Ur NAG, Ur NGAL, Ur OPN and Ur TP following exposure to a prototype chemotherapeutic known to induce dose-limiting nephrotoxicity across rodent and non-rodent models that are commonly used in preclinical drug development.

1672 Feasibility Study of Novel Kidney Safety Biomarkers in Male Beagle Dogs Following Cisplatin Administration: Assay Validations and Normal Baselines in Male and Female Dogs

Y. Chen[1], L. Smith[2], F. Almy[2], J. Ma[1], C. Lanigan[1], M. Otieno[2], S. Snook[1], M. Sonee[2] and J. E. McDuffie[1]. *[1]Janssen Research & Development, L.L.C., San Diego, CA and [2]Janssen Research & Development, L.L.C., Spring House, PA.*

Serum creatinine (sCr) and blood urea nitrogen (BUN) represent minimally-invasive, traditional, insensitive and non-specific biomarkers for predicting acute drug-induced kidney injury (DIKI). The gold-standard indicator for kidney dysfunction is remarkably decreased estimated glomerular filtration rate (eGFR). Eight urine based protein biomarkers have been qualified for the early prediction of DIKI in rats. Regulatory authorities continue to encourage pharmaceutical companies to conduct nonclinical and clinical exploratory studies to further evaluate the translational relevance of changes in DIKI biomarkers, including urinary (Ur) alpha GST, clusterin (CLU), cystatin C (CysC), kidney injury molecule 1 (KIM-1), N-acetyl-beta-D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), osteopontin (OPN), total protein (TP), and serum (s) CysC. To first identify dog-specific immunoassays for the evaluation of candidate DIKI biomarkers, a feasibility study was conducted in male beagle dogs. Animal received 5 consecutive once a day (D) intravenous infusions of the antineoplastic cisplatin (0.75 mg/

kg/day) or saline. Serum/urine samples were collected at pre-dose, D1-D6, D8, D10, and D15 (termination). Clinical observations included decreased body weight gain, no/soft stools, emesis, decreased food consumption, lethargy and dehydration. Cisplatin induced significant ($p<0.05$) increases in BUN, sCr and sCysC, Ur TP, Ur OPN, Ur KIM-1, Ur CLU, Ur CysC and Ur NGAL. Cisplatin-related minimal-to-moderate renal tubular lesions and immunopositive CLU, KIM-1 and OPN staining in damaged tubular epithelial cells were evident. We showed for the first time (1) a panel of fit-for-purpose validated dog-specific single-plex immunoassays; (2) a preliminary database of baseline DIKI biomarker levels in healthy male and female beagle dogs; and (3) progressive tubular damage associated with temporal changes in novel urinary DIKI biomarkers in cisplatin-treated male beagle dogs.

1673 Microfluidics and High-Content Imaging for *In Vitro* to *In Vivo* Safety and Efficacy Assessments

D. J. Sloan[1], J. Gordon[2] and R. McClelland[1]. [1]*SciKon Innovation, Chapel Hill, NC and* [2]*The Tauri Group, Frederick, MD.*

We evaluated the robustness and applicability of a newly designed microfluidic system, SciFlow, to support 2D and 3D high-content imaging via automated, fixed, and adaptable z-height focusing. Standard 2-dimensional static cell culture systems are limited in their predictive power for *in vitro* efficacy evaluations. Cells grown in static culture wells are subjected to increasing amounts of waste products and decreasing amounts of dissolved oxygen leading to potential stress responses and toxicity not related to the toxicant added. To overcome these limitations, researchers are turning to 3D scaffolds, spheroids, hydrogels, and fluidic systems to improve the physiological relevance of the environment and improve safety assessments. The SciFlow system sequentially links wells together to form a cascade of cell chambers through which drugs or toxicants can be applied. In order to enable efficient flow of fluids, well to well, the microfluidics are designed with descending well-heights from left-to-right so each subsequent well is 0.5mm lower than the previous well (i.e. z-height changes). Both hydrodynamic and capillary actions allow for cascading flow of fluids across the system without the need for external pumps or tubes. Toxicants interact with cells in the upstream compartments creating metabolites that will mix and interact in downstream wells forming a parent-metabolite gradient in a time-resolved fashion. One application for this technology is to visually image, in real-time, cellular responses during the movement of biological fluids, chemical compounds, and materials. We evaluated the cytotoxic effects of aflatoxin b, tamoxifen and acetaminophen on various metabolically competent hepatocyte models in comparison to static conditions using the reagent CellTox Green. Additionally, through a fluorescein tracer molecule, we could demonstrate that exposures in the fluidic system were non-linear and shaped similarly to an expected plasma curve *in vivo*. Time-resolved dynamic exposure scenarios are more *in vivo*-like and enable more accurate assessments of adaptive vs toxic mechanisms. Moreover, the dynamic exposure scenario allows better safety predictions of concentration thresholds than endpoint dose-response curves. Observation of shifts in the dose-response curves as a function of distance from source well provides an indicator for the role of metabolic activity in safety and efficacy assessments of these chemicals.

1674 Application of a Machine Learning Method to Identify Kinases Associated with Recurrent Hemodynamic Liabilities

R. Denton[3], S. Hnatyshyn[2], G. Thalody[1], J. Hennan[1], S. Ruepp[2] and P. Levesque[1]. [1]*Discovery Toxicology, Bristol-Myers Squibb, Hopewell, NJ;* [2]*Discovery Toxicology, Bristol-Myers Squibb, Lawrenceville, NJ and* [3]*Discovery Toxicology, Bristol-Myers Squibb, Wallingford, CT.*

Cardiovascular (CV) effects arising from off-target interactions are a likely reason for early kinase inhibitor developmental failures. Small molecule kinase inhibitors share promiscuous orthology for a variety of ATP binding sites across the kinome. Thus, shared liabilities for kinase inhibitors are common, and contribute to high CV failure rates. In order to better predict the potential for adverse CV outcomes, 76 individual molecules from neuroscience, cardiovascular, and oncology therapeutic areas were evaluated for interaction with 135 off-target human kinases. In parallel, aortic ring relaxation was measured using tensionometry at 0.1–30 uM test concentrations *ex vivo*. All data were analyzed with Random Forest Algorithm Machine Language Algorithm (RFA-MLA), a machine learning tool that enables a systems biology approach to the complex and high dimensional heterogenous data sets that were generated. A cut-off criteria for inclusion into recursive analysis was performed using rat telemetry on 12 candidate molecules *in vivo* demonstrating a representative range of aortic relaxation. Based on decreased mean arterial pressure (MAP) *in vivo*, an arbitrary 'bagging criteria' of >60% aortic dilation was used for selection into statistical analysis. Recursive RFA-MLA was used to correlate kinase inhibition with candidate targets presumed as contributing to aortic dilation *ex vivo*, and data relationships were assessed by linear regression analysis. Of ten kinases showing the highest statistical significance ($p< 0.04$), five candidate kinase targets were identified and validated by literature review as being known or associated with profound cardiovascular effects. These include CAMK, ROCK1, ROCK2 and PRKO2 (p140 rac/rho associated kinase) kinases. In the instances of ROCK1 and ROCK2, analysis of covariance (ANCOVA) was subsequently performed to exclude them from confounding associations with other kinase targets. From this covariance analysis, two additional kinases including NUAK2 and MLYK were identified. The kinases identified by this assessment are now included in early broad screening safety panel assessments.

1675 Application of a Shared Preclinical Database (eTOX) for Early Safety Assessment in Pharmaceutical Development (eTOX I)

T. Steger-Hartmann[2], F. Pognan[1] and M. Pawletta[2]. [1]*Discovery Investigative Safety, Novartis, Basel, Switzerland and* [2]*Investigational Toxicology, Bayer HealthCare, Berlin, Germany.*
Sponsor: F. Pognan.

In the framework of the European Innovative Medicines Initiative (IMI), 13 pharmaceutical companies extracted data from archived systemic toxicity studies and set up a database. The steadily growing database currently contains more than 8000 reports related to approx. 1400 investigated drug candidates. Due to its complex search capabilities, the database can be queried for structural similarity, similar target classes and specific toxicological endpoints. The database therefore represents an ideal tool to assess hit or lead structures during the early phases of drug discovery. In a feasibility trial we investigated the coverage of the database with regard to new drug discovery projects (n=10). Setting structural similarity to a threshold of 50% (Tanimoto coefficient), similar structures were detected in 6 out of ten projects. In 6 out of ten projects toxicity results could be found for the same target class in the data base. For three additional projects related targets, i.e. belonging to the same protein family were identified. The results contributed to the early assessment of candidates particularly with regard to distinction between target-related and off-target effects. The eTOX database therefore represents a valuable tool for early animal-free assessment of drug candidates.

1676 Predicting and Assessing the Risk of Drug Crystallization

S. Ruepp[4], E. Janovitz[1], T. Brodie[3], R. White[2], J. Hynes[4], J. Carman[4], D. Pan[3], C. Su[4], Y. Wu[5], U. Hanumegowda[5] and B. Gemzik[4]. [1]*Bristol-Myers Squibb, Hopewell, NJ;* [2]*Bristol-Myers Squibb, Mount Vernon, IN;* [3]*Bristol-Myers Squibb, New Brunswick, NJ;* [4]*Bristol-Myers Squibb, Princeton, NJ and* [5]*Bristol-Myers Squibb, Wallingford, CT.*

Low solubility is a common challenge in drug discovery that can often be overcome by formulation strategies. However, intrinsic low solubility remains a potential limitation that can impact early hazard identification. A novel aromatic substituted fluoroalkyl nicotinamide (AFAN) was advanced into toxicity studies despite low solubility (<1 µg/mL). Single oral dosages up to 300 mg/kg were well tolerated in rats, but tolerability deteriorated with repeated dosing with evidence of birefringent crystals and granulomatous inflammation in multiple organs at dosages ≥ 20 mg/kg/day (Cmax ≥ 35 µM). Raman spectroscopy confirmed the identity of the crystals as AFAN. Follow up investigations were conducted to determine what physicochemical properties contributed to drug crystallization in order to improve the ability to predict and assess the risk of crystallization *in vivo*. In cultured macrophages, AFAN (30 µM) formed crystals that were internalized by phagocytosis within 2 hours after formation, suggesting that these events likely precede granulomatous inflammation. Additional analyses confirmed that AFAN had the potential to form inter-molecular hydrogen bonds, with formation of very stable crystals and high crystal lattice energy. The melting temperature of AFAN (280°C) was also considered an important indicator of risk for *in vivo* crystallization. Collectively, the evidence from *in vitro* and *in vivo* studies indicated that low solubility along with high melting temperature and inter-molecular hydrogen bonding led to *in vivo* formation of AFAN crystals in a time-dependent manner. The extracellular drug crystals undergo phagocytosis with macrophage activation and subsequent granulomatous inflammation. These effects are not acute, but require repeat dosing up to 2 weeks in order to be identified.

1677 Exploiting the Diversity Outbred Mouse Model to Identify a Sensitive Preclinical Model and Underlying Mechanism of MK-0536 Induced Liver Injury

K. Pearson[1], T. Johnson[1], R. Gonzalez[1], L. LaFranco-Scheuch[1], R. Amin[1], R. Amin[1], W. Glaab[1], F. Sistare[1] and A. Harrill[2]. *[1]Merck, West Point, PA and [2]The University of Arkansas for Medical Sciences, Little Rock, AR.*

Predicting clinical drug-induced liver injury (DILI) from preclinical toxicology studies remains a challenge in drug development because preclinical models may not provide the same biological context that results in human DILI. New *in vitro* and *in vivo* tools are being developed to improve predictivity, but they tend to be focused on specific modes of action, and therefore, cannot cover all DILI scenarios. Additionally, without an appropriate preclinical animal model of compound-specific human DILI, effective recognition of molecules with improved toxicological properties is constrained. The Diversity Outbred (DO) mouse model offers a unique opportunity to investigate a failed compound's DILI potential across a broad landscape of genetic diversity within a relatively economical study design. We recently completed the conduct of a study with 100 female mice that were treated with either 1000 mg/kg/day MK-0536 or vehicle for 14 days (p.o.). Initial results indicate that 2 of the 50 treated mice presented with significant elevations in liver-specific biomarkers GLDH (2-3X) and miR-122 (approximately 10X). Biomarker elevations were associated with increased drug concentrations in the liver. These 2 animals had liver drug concentrations 2.5 and 4 times the mean liver concentration and approximately 10 and 70% higher than the animal with the next highest concentration, suggesting that increased tissue exposure in genetically predisposed individuals played a role in toxicity outcomes. In conclusion, use of a DO mouse population enabled identification of specific individuals that are genetically susceptible to MK-0536 DILI, thus providing an avenue that may enable mechanistic study of liver injury signals observed in clinical trials.

1678 Age-related Differences of Transporter Gene Expression in the Kidney of Rats

Y.-J. Xu, Y. Wang, J. Liu and Y.-F. Lu. *Zunyi Medical College, Zunyi, China.*

Kidney transporter proteins influence the disposition of Endogenous substances and xenobiotics by participating in absorption, distribution, and elimination. Renal transporters also help maintain electrolyte homeostasis, provide organ/tissue defense, and contribute to the therapeutic effects and toxicity of many drugs and toxicants. Many factors could influence kidney transporter's expression and affect drug disposition, efficacy and toxicity. This study was aimed to examine the development- and aging-associated variations in major renal transporters in rats including the transporters responsible for renal uptake of xenobiotics such as organic anion transporter (Oat1, Oat3), organic cation transporter (Oct1, Oct2, Oct3), and organic anion transporting peptide (Oatp4c1), as well as transporters related to kidney efflux and excretion of xenobiotics such as multidrug resistant protein (Mdr1), breast cancer resistance protein (BCRP/ABCG2), multidrug resistance associated proteins (Mrp2, Mrp4), Multidrug and toxin extrusion protein (Mate1, Mate2-K). The kidneys from SD rats during development (-2, 1, 7, 14, 21d), maturation (28, 35, and 60 d) and aging (180, 540 and 800 d) were collected and total RNAs were extracted, purified, and subjected to real-time RT-PCR analysis. Total proteins were also extracted for western-blot analysis of interested proteins. Results showed that Oat1, Oat3, Oct1, Bcrp, Mrp2, Mrp4 and Mate2-k was low in fetal kidneys and increased gradually after birth, and dramatically increased after maturation and adulthood (180 d), and maintained at that high level till 850d of the age. Oct2, Oatp4c1, Mdr1b and Mate1 was low in fetal kidneys and increased gradually after birth, and increased dramatically after weanling, maturation and adulthood (180d), but it was decreased at aging (850d). Oct3 mRNA expression was low in fetal kidneys and newborn kidneys, and had two peaks at 35d and 850d. The selected protein expression showed the similar expression trends. Thus, kidney transporter expressions are affected by ontogeny and aging, which could impact drug and toxicant disposition in children and elderly.

1679 Analysis of Ingredient Lists to Quantitatively Characterize Chemicals in Consumer Products

K. K. Isaacs[3], K. Phillips[1,3], J. Wambaugh[3] and P. S. Price[3]. *[1]Oak Ridge Institute for Science and Education, Research Triangle Park, NC; [2]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC and [3]National Exposure Research Laboratory, US EPA, Research Triangle Park, NC.*

The EPA's ExpoCast program is developing high throughput (HT) approaches to generate exposure estimates to compare against HT bioactivity data generated by the US inter-agency Tox21 and the US EPA ToxCast programs. Assessing exposures for the thousands of chemicals in consumer products requires data on product composition. This is a challenge since quantitative product composition data are rarely available. We developed methods to predict the weight fractions of chemicals in consumer products from weight fraction-ordered ingredient lists, and curated a library of such lists from online manufacturer and retailer sites. The probabilistic model predicts weight fraction as a function of the total number of reported ingredients, the rank of the ingredient in the list, the minimum weight fraction for which ingredients were reported, and the total weight fraction of unreported ingredients. Predicted weight fractions compared very well to available quantitative data obtained from Material Safety Data Sheets for products with 3-8 ingredients. Lists were collected from the online sources for 5148 products containing 8422 unique ingredient names. A total of 1100 of these names could be located in EPA's HT chemical database (DSSTox), and linked to 864 unique Chemical Abstract Service Registration Numbers (392 of which were in the Tox21 chemical library). Weight fractions were estimated for these 864 CASRN. Using an EPA database of chemical function, the functions associated with the largest numbers of chemicals in this dataset were surfactants (N=278), skin conditioners (N=258), and fragrances (N=251). Functions found in the highest number of products were viscosity controllers (N=2906), solvents (N=2737) and preservatives (N=1745). Functions with the highest mean predicted weight fractions were fillers (0.15±0.24), antimicrobials (0.09±0.18), masking agents (0.09±0.19), and UV absorbers (0.08±0.16). These ingredient data sources and weight-fraction prediction models advance our ability to parameterize empirical and mechanistic exposure models for chemicals in consumer products in support of risk-based decision-making. This abstract does not necessarily reflect US EPA policy.

1680 Qualitative Structure-Property Relationships for Predicting Chemical Functional Use and Weight Fractions in Consumer Articles

K. Phillips[2,4], J. Wambaugh[4], P. Egeghy[4], C. Grulke[1] and K. Isaacs[4]. *[1]Lockheed Martin, Research Triangle Park, NC; [2]Oak Ridge Institute for Science and Education, Oak Ridge, TN; [3]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC and [4]National Exposure Research Laboratory, US EPA, Research Triangle Park, NC.*

Chemical functional use—the functional role a chemical plays in processes or products—may be a useful heuristic for predicting human exposure potential in that it comprises information about the compound's likely physical properties and the product formulations or articles in which it is likely to be found. Functional use is also an integral part of alternatives assessment, which involves identifying safer chemicals that can perform the same role in products as current chemicals. Using publicly available information, we have compiled a data set 14,000 chemicals and 240 functional uses. This data set was used to build classifier models for 49 functional use categories using chemical structures and physical properties which were described using ToxPrint chemical descriptors and the US Environmental Protection Agency's Estimation Program Interface Suite, respectively. Separately, data were collected from the Danish EPA, which provides concentrations of over 300 chemicals across 202 unique articles. Using the functional use predicted from the classifier models and structure and physical property descriptors, models were constructed to predict the mass fraction of chemicals in household articles. An external validation set of chemicals reported by the state of Washington's Children's Safe Product Act was used to compare model results with reported concentrations. The models developed here will be useful in developing and parameterizing high-throughput models of human exposures for chemicals for which little measured or reported data are available. The views expressed herein are those of the authors and do not necessarily reflect the views or policies of the US EPA.

1681 EPA's Multiple-Strategy Approach for Characterizing the Composition of Consumer Products

P. Price, K. Isaac, K. Phillips and J. Wambaugh. *Office of Research and Development, US EPA, Durham, NC.*

Commercial products have been identified as significant sources of exposure for many chemicals. Estimating such exposures is a challenge because data on composition are often not available and because individuals use a range of products (i.e. brands) with varying formulae. (Data on composition are limited because manufactures may not know the composition of the components of their products or compositions are proprietary.) EPA is using a multi-pronged approach to characterizing composition of products. First, EPA is searching the internet for information on product composition. Sources of such data are manufactures who have chosen to make such data public and composition data from material safety data sheets. Data on composition of 6627 products containing 2346 chemicals have been identified. Second, EPA has developed a method of estimating composition when an ingredient list is available for a product. Such estimates are possible because ingredients are listed in order of weight fraction. Ingredient lists are available for more than 5148 products containing approximately 7869 unique ingredients. These approaches, however, do not cover the majority of the substances currently in commerce. A third approach, which can be applied to chemicals with unknown uses, is to use information on the function of a chemical in a product. Physical/chemical characteristics of chemicals that are known to provide specific functions in a class of products are used to create models that predict 1) the functions that a chemical could provide in different types of products and 2) the weight fraction required. Finally, EPA is analyzing selected consumer products using advance analytical techniques which can identify a wide range of chemicals. The results of these efforts will be a tool that consists of databases of product formulae and models to predict chemicals without data. Where multiple formulae are available for various classes of products, this information will be used in probabilistic models of exposure to characterize inter- and intra-individual variation in exposure. This abstract does not necessarily reflect US EPA policy.-

1682 Product Deformulation to Identify Exposure Pathways for ToxCast Chemicals

J. Wambaugh[4], A. Yau[3], K. Phillips[2,4], K. A. Favela[3], C. Nicolas[2,4], D. Biryol[2,4], C. Grulke[1,4], A. Richard[4], P. Price[4], A. Williams[4], K. Isaacs[4] and R. Thomas[4]. *[1]Lockheed Martin, Research Triangle Park, NC; [2]Oak Ridge Institute for Science and Education, Oak Ridge, TN; [3]Southwest Research Institute, San Antonio, TX and [4]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC; [5]National Exposure Research Laboratory, US EPA, Research Triangle Park, NC.*

The health risks posed by the thousands of chemicals in our environment depends on both chemical hazard and exposure. However, relatively few chemicals have estimates of exposure intake, limiting risk estimations. We have previously developed a heuristics-based exposure estimation method that depends on simple factors such as the presence or absence in consumer products to estimate exposure for thousands of chemicals. Although this method is predictive when compared with human biomonitoring data, limited data are available on the chemical constituents within most articles of commerce. We aim to broaden the number of products with constituent chemical information and refine exposure models by quantifying constituent concentration. A selection of five samples each from 20 diverse consumer product categories (e.g., lotion, clothing, carpet) were analyzed using solvent extraction followed by gas chromatograph (GC) x GC Time of Flight Mass Spectrometry (GCxGC-TOF/MS), which is suited for forensic investigation of complex matrices. In an examination of five plastic children's toys, a total of 306 unique compounds were identified across all toys, including 102 Tox21 chemicals such as diethyl phthalate and bisphenol AF. As many as 114 and as few as 56 chemicals were identified in each toy. Across the five toys, a range of 0 to 40 unique peaks remain unidentified. Interestingly, bisphenol A (BPA) was identified in a toy marked as "BPA free", demonstrating the possibility of the unexpected presence of chemicals in some products. In addition to characterizing more products, we are working to identify those GCxGC-TOF/MS signatures that are currently not linked to known chemicals by broadening the library of retention times and spectra for likely suspect chemicals among the Tox21 chemical library. The qualitative and quantitative information developed from this project will be used to calculate and refine exposure estimates for the thousands of chemicals of chemicals in articles and consumer products. This abstract does not necessarily reflect U.S. EPA policy

1683 Using ToxPi to Determine Biological Activity and Toxicity of Chemicals Present at Superfund Sites in North Carolina

S. K. Tilley[2], A. Nguyen[2], K. To[1], D. M. Reif[1] and R. C. Fry[2]. *[1]Bioinformatics Research Center, North Carolina State University, Raleigh, NC and [2]Environmental Health Science and Engineering, Gillings School of Global Public Health, Chapel Hill, NC.*

The Superfund program of the Environmental Protection Agency (EPA) was established in 1980 to address public health concerns posed by toxic substances released into the environment in the United States. There are approximately 40 designated Superfund sites in the state of North Carolina. We set out to develop a database that housed information on both the prevalence and biological activity/toxicity of chemicals present at Superfund sites in North Carolina. The information on chemicals present at Superfund sites was gathered through publicly available information through the EPA. We then used a toxicity-ranking tool, the Toxicological Priority Index (ToxPi), to determine the biological activity and toxicity of a subset of these chemicals included in the ToxCast and Tox21 programs. Through this integration, we have identified that many highly-ranked chemicals are not contained within the ToxCast and Tox21 datasets. From all chemicals that could be queried within our database, we present ToxPi analyses to rank their biological activity/toxicity. Our data not only represent a novel profile of the most prevalent chemicals at North Carolina Superfund sites, but also provide critical information on the potential modes of actions of some of these chemicals.

1684 Comparative Study of Potential Ambient Air Exposures to Residents Living Near Contaminated Sites

C. DeHate[2], R. DeHate[2], G. Johnson[2], B. Skelly[1] and R. Harbison[2]. *[1]GEI Consultants, Valrico, FL and [2]Environmental Occupational Health, College of Public Health, Tampa, FL.*

According to the United States Environmental Protection Agency (EPA) approximately 11 million people in the US. live within 1 mile of a federal Superfund site; including an estimated 3–4 million children. When you include the number of people living near contaminated sites under State jurisdiction, this number has been estimated to be as high as 49 million people presented with potential public health risks. In this study we looked at whether people living near contaminated sites were at a greater health risk from potential ambient air exposures. In 2001 the EPA published the results of an indoor air and outdoor air quality study, Building Assessment and Survey Evaluation (BASE), conducted at randomly selected public and commercial office buildings across the U.S. In addition, the New York State Department of Health (NYSDOH) conducted a study of indoor and outdoor air of homes heated with fuel oil. None of the sites in these studies were located near a contaminated site. Although the focus of both of these studies was indoor air they both supply comparative background data for outdoor air. We collected outdoor air samples from 115 locations from residential and commercial properties adjacent to contaminated sites and compared our data to these background data sets. We collected a total of 435 outdoor air samples that were analyzed for volatile organic compounds using EPA Method TO-14A and TO-15. The background data sets' method reporting limits for non-detects ranged from 0.35 to 58 µg/m3. Our data set was then compared to the minimum, maximum, means and the 25th, 50th, 75th, 90th, 95th and 99th percentile background values. Our results show no significant differences between ambient air values in the background data sets to the values in our study concluding, in this limited study, that ambient air did not present a significant risk to people living near contaminated sites.

1685 Chemical Emissions via Smoke Produced Through Burning of Scrap Tires, Firewood and Liquefied Petroleum Gas as Fuel Sources for Singeing Meat in Ghana

E. Afriyie-Gyawu[4], A. Brown[4], R. Barham[1] and H. Rochani[4]. *[1]Erskine College, Due West, SC; [2]Biostatistics, Georgia Southern University, Statesboro, GA; [3]Environmental Health, Georgia Southern University, Statesboro, GA and [4]Environmental Health Sciences, Georgia Southern University, Statesboro, GA.*

This pilot study aims at determining the presence and levels of toxic chemicals emitted through smoke produced via burning of scrap tires, firewood, and liquefied petroleum gas (LPG) used as fuels for singeing meat intended for humans. Burning of these fuels reportedly emits carbon monoxide (CO), benzene, and other air pollutants via the smoke. CO decreases the carrying capacity of blood to transport oxygen to the

organs, and can ultimately lead to death. Benzene is associated with irritation of the skin, eyes, and airways; vomiting, dizziness, etc.; and it is a known human carcinogen. Concentrations of these pollutants were quantified using a chip measurement system (CMS) from Draeger Safety, Inc. Analysis was conducted at six different locations in four major cities in Ghana, where animal carcasses are processed for human consumption. Four locations (slaughterhouses) use tire-derived fuel, the fifth (mechanized abattoir) uses LPG, while the sixth uses firewood (with a small piece of tire to start the fire) for meat singeing. Overall, the average CO levels emitted via tire-derived smoke (128.67±18.23 ppm; $p<0.0001$) and firewood smoke (130.86±21.71 ppm; $p<0.0001$) were significantly higher compared to LPG-based smoke (control). No significant difference was detected between the level of CO in tire-derived smoke versus that of firewood ($p<0.8915$). Also, the average benzene levels emitted via scrap tire-derived smoke (3.50±0.95 ppm; $p<0.2163$) and firewood (1.30±1.14 ppm; $p<0.8712$) were higher than that of LPG-based smoke (control) but not statistically significant. Also, there was no significant difference between level of benzene in tire-derived smoke versus that of firewood ($p<0.1053$). While this is a pilot study, results suggest that slaughterhouse operators and residents in close proximity to these facilities may be at high risk of experiencing the health effects of CO and benzene. Further studies are needed to confirm these results and work with stakeholders to create data-driven, safe, economically viable, and culturally appropriate alternatives for processing meat in Ghana and other developing countries.

PS 1686 Factors Affecting Hydrocarbon Gas and Vapor Exposure of Upstream Oil and Gas Workers during Completion and Production Activities at Unconventional Shale Oil and Gas Wells

J. E. Snawder[1], M. Alexander-Scott[1], M. J. Breitenstein[1], E. J. Esswein[2], B. C. Johnson[1], B. King[2], C. A. Striley[1] and J. Toseski[1]. [1]*National Institute for Occupational Safety and Health, Cincinnati, OH and* [2]*National Institute for Occupational Safety and Health, Denver, CO.*

The National Institute for Occupational Safety and Health is conducting research to assess chemical exposure hazards to upstream oil and gas workers. Activities in each segment may present chemical exposure hazards inherent to that activity and vary based on well type, geologic basin and the age of the producing well. Worker exposures were determined by personal breathing zone (PBZ) sampling during the full-shift or for specific tasks. Exposure assessments included: low molecular weight gas and vapors, benzene, toluene, ethylbenzene, xylenes (BTEX), and polyaromatic hydrocarbons (PAHs). Identification of exposure sources and patterns of exposure were evaluated through collection of whole air samples (grab and time integrated samples), direct reading instruments including flame and photoionization detectors, colorimetric methods and infrared videography. Predictably, alkanes, alkenes, BTEX and PAHs (particularly naphthalene) were detected in many PBZ samples; however, the composition and concentration of different hydrocarbons varied greatly. Well completion activities presenting the greatest hydrocarbon exposures included flowback, production testing and produced water management; production activities included tank gauging and sample collection during fluid transfers, well inspection and maintenance. Geologic basin, well type, age or stage of production were determined to be predictors of both the composition and concentration of hydrocarbon exposures Higher concentrations of BTEX were detected in worker samples from oil and gas wells in DJ, Piceance, San Juan, and Bakken formations, followed by wet gas wells in Utica and Marcellus basins. Worker samples from dry gas wells in Fayetteville and Marcellus basins had low concentrations of the measured hydrocarbons and when detected were often from sources other than the well. Geologic formation, worker activities, age of the well and design of the production equipment were all factors that effected exposures. Greater understanding of where, when and how exposures occur can allow for better risk assessments and targeted interventions and controls to improve worker safety and health.

PS 1687 Impact on the Respiratory System Due to Travel to Cities Abroad–A Focus on Changing Air Pollution Levels

M. J. R. Vilcassim, L. C. Chen and T. Gordon. *Environmental Medicine, New York University, Tuxedo, NY.*

Many large cities in the world are known to have many sources of air pollution and thus elevated pollutant levels, especially during certain environmental conditions. These higher levels of air pollution have been shown to have adverse respiratory impacts on residents living in such cities. Although previous studies have looked at health effects of change in air pollution on residents, no studies have looked into the impact on the respiratory and cardiovascular system when individuals travel to cities abroad with air pollution levels different to their residing city. Therefore, this study's goal is to understand the impact on the respiratory system due to a change in the environment when traveling to a new city in another country, with a main focus on changing air pollution levels. The study recruited students traveling from NYC to cities abroad, mainly those who are enrolled in the NYU Study Abroad program. Three main cities where students traveled - Geneva, Prague and Mexico City were selected as study sites in this pilot study. Participants were pre-trained to use a portable spirometer which recorded FEV1 and PEF measurements before departure, during the stay and after returning from the city abroad. Participants also carried a portable PM2.5 monitoring instrument, an 'Airbeam', to measure their personal exposure levels. Results from the pilot study have shown that in students who traveled to Geneva and Prague, there was no major change in lung function - with the change in exposure. The pollution level changes were not significant for either city vs NYC. In students who traveled to Mexico City there was a noticeable decrease in lung function with increasing pollution. The average reduction in FEV1 was 6–9% during the initial week in Mexico City while upon return to NYC an increase of 11–15% was recorded in average FEV1 values. The significant change in PM2.5 levels between the two cities was correlated with the change in lung function. Therefore, the results of this pilot study show that travel to cities with higher pollution can have acute impacts on the respiratory system and support a concern for the potential for adverse impacts due to exposure to varying air pollution in travel to cities abroad.

PS 1688 Relationships Between Number, Surface Area, and Mass Concentrations of Different Nanoparticles in Workplaces

M. Zhang[2], Q. Zhang[1] and H. Zou[2]. [1]*Department of Environmental and Occupational Health Sciences, University of Louisville, Louisville, KY and* [2]*Environmental and Occupational Health, Zhejiang Province Center for Disease Control and Prevention, Hang zhou, China.*

No consistent metric for measuring exposure to nanoparticles has yet been agreed upon internationally. This study seeks to examine the relationship between number concentration (NC), surface area concentration (SAC), and mass concentration (MC) of nanoparticles in workplaces. Real-time NC20-1000nm, SAC10-1000nm, and respirable MC100-1000nm were determined for different nanoparticles. Concentration ratio (CR, activity: background), exposure ranking (ER), and between-metric correlation coefficients (R) were used to analyze the relationships between the three metrics. The ratio of cumulative percentage by number (APN) and cumulative percentage by mass (APM) was used to analyze whether nanoparticle number is predominant, as compared with nanoparticle mass. The CRs of NC20-1000nm and SAC10-1000nm for different nanoparticles at the corresponding work sites were higher than those of respirable MC100-1000nm. The ERs of NC20-1000nm for nano-Fe2O3 and nano-Al2O3 were the same as those of SAC10-1000nm, but were inconsistent with those of respirable MC100-1000nm. The order of correlation coefficients between NC20-1000nm, SAC10-1000nm, and respirable MC100-1000nm was: RSAC and NC > RSAC and MC > RNC and MC. The ratios of APN and APM for nano-Al2O3 and grinding-wheel particles (less than 100 nm) at the same work site were 2.03 and 1.65, respectively. NC and SAC metrics are significantly distinct from MC in characterizing exposure to airborne nanoparticles. Simultaneous measurement of NC, SAC, and MC should be conducted as part of nanoparticle exposure assessment strategies and epidemiological studies. Keywords: Nanoparticles; Exposure metric; Exposure assessment; Workplace

PS 1689 Assessment of Indoor Formaldehyde Concentrations Following the Installation and Removal of Laminate Flooring

J. S. Pierce[1], A. Abelmann[1], P. S. Ruestow[1], J. T. Lotter[1], E. M. Beckett[1], H. A. Fritz[1], J. L. Bare[1] and K. M. Unice[2]. [1]*Cardno ChemRisk, Chicago, IL and* [2]*Cardno ChemRisk, Pittsburgh, PA.*

Objective: Concerns have been raised regarding formaldehyde emissions from laminate flooring. Given the limited sampling data available, an investigation of the potential formaldehyde emissions resulting from the installation, use, and removal of laminate flooring was conducted. Methods: Two laminate flooring products were purchased and installed in separate study rooms. Passive 24-hr diffusive badge samples (n = 79) for formaldehyde were collected over 63 days, including during a pre-installation period, an acclimation period, during which the packaged products were stored in the study rooms, and following installation and removal. The concentrations were compared to exposure limits and guidelines that exist in the U.S. for indoor air. Results: Mean background

concentrations were 0.006 ppm and 0.005 ppm in Rooms 1 (R1) and 2 (R2), respectively. During acclimation, mean concentrations increased to 0.009 ppm and 0.010 ppm in the two rooms, respectively. Mean concentrations following the installation of the flooring were 0.038 ppm (range: 0.020-0.058 ppm) in R1 and 0.022 ppm (range: 0.013-0.039 ppm) in R2; these concentrations were statistically significantly higher than background ($p < 0.001$) and acclimation concentrations (R1: $p < 0.001$; R2: $p = 0.011$). Upon removal of the flooring, concentrations decreased rapidly and, on post-removal day 7, were not statistically significantly elevated compared to background. Throughout the study (including prior to and during acclimation), mean airborne concentrations exceeded the 8-hr and chronic Reference Exposure Level (REL) of 0.007 ppm and the Proposition 65 No Significant Risk Level (NSRL) of 40 µg/day (24-hr equivalent: 0.002-0.003 ppm) set by California's Office of Environmental Health Hazard Assessment (Cal-OEHHA). Following the installation of the flooring, the Federal Emergency Management Agency (FEMA) procurement standard (0.016 ppm) was also exceeded in both rooms. Conclusions: Formaldehyde was detected in indoor air at levels that exceeded background during the acclimation of the products, following flooring installation, and up to 7 days following removal. Concentrations resulting from the use of both products exceeded non-occupational indoor air exposure limits and guidelines in the US during all study periods.

1690 Historical Ambient Airborne Asbestos Concentrations in the United States—An Analysis of Published and Unpublished Literature

A. Abelmann[1], M. E. Glynn[1], J. S. Pierce[1], P. K. Scott[2], S. Serrano[3] and D. J. Paustenbach[3]. *[1]Cardno ChemRisk, Chicago, IL; [2]Cardno ChemRisk, Pittsburgh, PA and [3]Cardno ChemRisk, San Francisco, CA.*

Outdoor concentrations of airborne asbestos have been measured throughout the US over time. However, a thorough review and analysis of these data has not been conducted. The purpose of this study is to characterize asbestos concentrations in ambient air by environment type (i.e., urban, rural) and by decade, using measurements collected in the absence of known asbestos emission sources. A total of 17 published and unpublished studies and datasets were identified that reported the results of 2058 samples collected from the 1960s through the 2000s across the US. Most studies did not report asbestos fiber type, and data based on different analytical methods (e.g., Phase Contrast Microscopy, Transmission Electron Microscopy, etc.) were combined in the dataset; however only fibers ≥ 5µm in length were considered. For a small subset of the measurements (n = 186), a conversion factor was used to convert mass-based data (e.g., ng/m3) to count-based values (i.e., f/cc ≥ 5µm). The estimated overall mean and median ambient asbestos concentrations were 0.00093 f/cc and 0.00022 f/cc, respectively, and concentrations generally increased from the 1960s through the 1980s, after which they declined considerably. While asbestos use decreased throughout the 1970s, results indicate that ambient concentrations peaked during the early 1980s, which suggests the possible contribution of abatement or demolition activities. Lastly, ambient asbestos concentrations were higher in urban than rural settings which is consistent with the greater use of asbestos-containing materials in more densely populated areas.

1691 Prevalence and Health Risk From Exposure to Naturally Occurring Arsenic and Uranium in Well Water in Connecticut

S. M. Rusnak. *Environmental and Occupational Health Assessment Program, Connecticut Department of Public Health, Hartford, CT.*

Over the past three years, the Connecticut Department of Public Health (CT DPH) has sampled private wells throughout Connecticut for naturally occurring uranium and arsenic. With over 1140 well samples planned, CT DPH has tested about 800 wells to date. Uranium is much more prevalent than arsenic, but both contaminants have been found in wells throughout the state. Approximately 27% of wells tested have detectable levels of uranium and 4% have levels above state drinking water guidelines. Approximately 3% of the wells tested have arsenic present with 1% exceeding state drinking water guidelines. The maximum concentrations of uranium and arsenic in wells are 1420 and 120 parts per billion (ppb), respectively, and 47 and 12 times the state drinking water guidelines. Using the maximum level for arsenic, the lifetime cancer risk from drinking the contaminated water is to 2 excess cancer cases out of an exposed population of 1,000. While uranium isn't carcinogenic, exposure to high levels can lead to kidney damage. CT DPH is leading in a state wide effort to educate well water owners about the need to test their wells for arsenic and uranium and about health concerns related to the contamination. While CT DPH recommends private well testing for uranium and arsenic, there are no state or local requirements. The prevalence of naturally occurring arsenic and uranium raises broader questions about health risks related to exposure to naturally occurring contaminants in well water and the need for well testing.

1692 Dietary Intake of Perfluorooctane Sulfonate (PFOS) and Implications for Drinking Water Limits

H. N. Lynch, L. E. Kerper, M. Seeley and B. D. Beck. *Gradient, Cambridge, MA.*

PFOS exposure in the general population is widespread but gradually declining due to the large-scale phase out of PFOS manufacture beginning in the early 2000s. Estimated PFOS intake from all sources is relatively low, on the order of 0.01 µg/kg-day or less. Exposure sources for the general population include food, drinking water, and house dust, with diet as the largest source. Several agencies in the US and abroad have established daily intake guidelines. Recently, both the United States Environmental Protection Agency (US EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR) proposed values of 0.03 µg/kg-day; the UK Tolerable Daily Intake is 0.3 µg/kg-day. Based on these values, agencies have also set drinking water guidelines. These guidelines use either 10 or 20% as default values for the relative source contribution (RSC) from drinking water. Applying these default RSC values, in addition to relying on very conservative toxicological endpoints, likely yields drinking water guidelines that are unnecessarily conservative. We assessed the relative intake of PFOS from water, food, and other sources to establish alternative RSCs for PFOS. We reviewed data on estimated intake of PFOS from food and other sources and reviewed the assumptions used as the basis for the drinking water guidelines for PFOS. Given the relatively small total intake of PFOS from food (approximately 0.005 µg/kg-day or less), current drinking water guidelines are likely to overestimate the relative contribution from food and other sources and thus underestimate the RSC. Using the UK drinking water guideline as an example, we demonstrate that the use of more scientifically supported RSC values would yield health protective drinking water guidelines that are more than four fold higher than the current guideline.

1693 Estimate of 4-Methylcyclohexanemethanol (MCHM) Exposure with Normal Use of Contaminated Water During the Elk River Spill

R. M. Novick[3], P. K. Scott[2], B. Winans[3], S. M. Green[4] and D. J. Paustenbach[1]. *[1]Cardno ChemRisk, Jackson, WY; [2]Cardno ChemRisk, Pittsburgh, PA; [3]Cardno ChemRisk, San Francisco, CA and [4]Eastman Chemical Company, Kingsport, TN.*

Approximately 10,000 gallons of a mixture containing 90% crude MCHM were accidently released into the Elk River, West Virginia in January 2014. The spill occurred upstream of a water treatment plant, and contaminated water was distributed to the region. In this study, a screening-level exposure assessment was performed to estimate the adult daily dose of MCHM, the most abundant constituent of the crude material, from normal usage of contaminated water via ingestion, dermal exposure during bathing, and inhalation during showering. The estimate was performed at three MCHM water concentrations: the approximate analytical detection limit (0.01 ppm), the CDC drinking water advisory level (1 ppm), and the highest measured concentration in treated water (3.1 ppm). If the water was used normally at MCHM concentrations of 0.01, 1, or 3.1 ppm, the adult MCHM doses would be 3.38 x 10-4, 0.0338, and 0.106 mg/kg-d, respectively. At all three concentrations, 91.6% of the dose is due to the ingestion of drinking water, 7.45% of the dose is from dermal contact during showering or bathing, and 0.95% of the dose is from inhalation during showering or bathing. These results are compared to a recently published exposure assessment that was based on the Henry's Law Constant for MCHM and relied on different exposure factors. In both analyses, the estimates are certainly much higher than what occurred because MCHM has a strong odor at these concentrations and the residents were instructed not to use the water. The predicted exposures are several orders of magnitude lower than the no effect level in animal studies.

1694 Use of the Risk21 Framework to Determine Adequacy of Various Data Streams for Exposure Modeling in Risk Assessments

P. M. Hinderliter[3], W. Chen[3], C. C. Truman[3] and K. D. Yi[3]. *[1]Environmental Fate & Exposure, Syngenta Crop Protection, LLC, Greensboro, NC; [2]Product Metabolism, Syngenta Crop Protection, LLC, Greensboro, NC and [3]Toxicology and Health Sciences, Syngenta Crop Protection, LLC, Greensboro, NC.*

Risk assessment of pesticide exposures to humans is characterized by a large body of mammalian toxicological data and estimated exposures. Exposures are estimated from a combination of scenarios in which a population could be exposed (dietary intake, drinking water intake, and/or residential handling). Dietary exposure via residues on food is usually the most prevalent exposure scenario; however, for certain pesticides, drinking water can be the major route of exposure. Drinking water monitoring is not only costly, but the question of sampling frequency is also important. For widely used pesticides like atrazine, extensive water monitoring including daily/near daily samples may be available; however, for most pesticides monitoring data are very limited. Various methods have been used to fill the non-sampled days (filling the gaps) to allow for better temporal resolution for risk assessments. Using the daily/near daily monitoring data of atrazine, we show the performance of various methods (linear interpolation, statistically based bias factor and kriging) by evaluating the uncertainties (deviation from the true) of various estimates of assessment endpoints with specified likelihood (i.e., to minimize false negatives). For the atrazine case study (using these data and the existing toxicological data for atrazine), the Risk21 analysis tool was used to show that daily/near daily monitoring is not necessary, and that the kriging-predicted daily exposures are robust and provide sufficient resolution for risk assessment. This case study shows that the Risk21 framework is a useful and important tool in determining whether a sufficiently robust dataset is available for risk assessment and regulatory decision making.

1695 Soil Concentrations of Environmental Contaminants at Urban Agricultural Sites in New Orleans, Louisiana

K. M. Moller, J. Hartwell, B. R. Simon-Friedt, M. Wilson and J. K. Wickliffe. *Global Environmental Health Sciences, Tulane University, New Orleans, LA.*

Urban agriculture produces numerous social and economic benefits to the cities where it is practiced. However, these cities may have soils with unsafe levels of contaminants that could be unhealthy for farmers and gardeners and impact the safety of the produce grown in these areas. We are evaluating soil levels of 22 different elements at urban farms and gardens in New Orleans, LA to determine whether unsafe levels are present. Elements were analyzed by ICP/MS (Hg CVAAS) and field-portable X-ray fluorescence spectrometry (XRF). Results were benchmarked using the Louisiana Department of Environmental Quality's (LDEQ) soil standards. Analysis of 27 sites found that the XRF regularly reported higher concentrations of contaminants across multiple elements and was poorly correlated with ICP/MS measurements. For lead, however, XRF and ICP/MS measurements were highly correlated (Spearman's R = 0.82). A health risk assessment for the urban agriculture community is currently being developed. Ultimately, our objective is to inform both policy and plan, from sampling to mitigation, for examining soils in highly urbanized areas where legacy pollution may create a health hazard. Comparing the XRF to the EPA standard ICP/MS analysis method is one step in evaluating screening tools for practitioners of urban agriculture.

1696 Risks Assessment of Total Petroleum Hydrocarbon (TPH) in Four Important Seafood from Crude Oil Polluted Waters of Ogoniland, Rivers State, Nigeria

K. W. Nkpaa and M. O. Wegwu. *Department of Biochemistry, University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria.*

This study was designed to evaluate the risk levels of Total Petroleum Hydrocarbon (TPH) in seafoods from crude oil polluted waters of Ogoniland, Rivers State, Nigeria. GC-FID was used for the quantification technique. The result showed significant differences ($p \leq 0.05$) between TPH levels in seafood from Kaa/B-Dere and Kaa/Bodo City for Tilapia queneesis, Liza falcipinis and Callinectes pali. T. queneesis recorded cumulative TPH levels of 124±0.71, 1,048±26.6 and 1,549±2.01 µgkg-1 wet wt. at Kaa, B-Dere and Bodo City respectively, while L. falcipinis recorded cumulative TPH levels of 105 ± 0.03, 1,100 ± 6.10 and 1,610 ± 5.89 µgkg-1 wet wt. at Kaa, B-Dere and Bodo City respectively. Also, C. pali recorded cumulative TPH levels of 72.8±0.67, 746±9.57 and 1,201±16.7 µgkg-1 wet wt. at Kaa, B-Dere and Bodo City respectively while Penaeus notialis recorded cumulative TPH levels of 50.8±0.63, 365±10.3 and 1,031±12.5 µgkg-1 wet wt. at Kaa, B-Dere and Bodo City respectively. Between the three sites and the seafood analyzed, L. falcipinis (1,610±5.89 µgkg-1 wet wt.) accumulated significantly higher levels ($p<0.05$) of TPH at Bodo City. Accumulation of petroleum hydrocarbon may reduce the quality of this seafood tissues and cause tenting. Total petroleum hydrocarbon levels in most of the seafood tissue were at critical level (exceeding DPR set limits) and also suggest that these samples tissues may act as bioindicators of exposure owing to expressed contaminants uptake pattern. Possible health risk consequences of such high bioaccumulation of TPH on public health cannot be ignored.

1697 Polybrominated Diphenyl Ethers (PBDE) and Their Hydroxlyated and Methoxylated Derivatives in Seafood and Dust Collected From the Puget Sound, Washington Region

S. Cade, L.-J. Kuo and I. Schultz. *Pacific NW National Lab, Sequim, WA.*

The most important routes of human exposure to PBDEs are from contaminated food and contact with dust found in households and workplaces. Structurally related derivatives of PBDEs are the hydroxylated (OH-PBDEs) and methoxylated forms (MeO-PBDEs). Humans can metabolize some PBDEs into the hydroxylated forms, which is a concern due to greater health risks associated with OH-PBDEs. However, certain OH-PBDEs and MeO-PBDEs are also marine natural products and it is unclear although likely, that marine fish and shellfish, which bioaccumulate these compounds serve as a vector for human exposures. In this study, we measured approximately 30 different PBDE, OH-PBDEs and MeO-PBDEs in edible shellfish and finfish collected or purchased from seafood markets and restaurants located in the Puget Sound region of Washington State. We also measured 12 common PBDE congeners including PBDE-209 in dust samples collected from electronic waste (E-waste) recycling businesses, non-E-waste businesses and private homes. The focus on E-waste recycling was due to concerns that it's an occupation that may expose workers to dust with enriched levels of PBDEs. Results from the dust analysis indicated the predominant PBDE congeners measured were: PBDE-209 > PBDE-153 >> PBDE-183 > PBDE-47 > PBDE-99 > PBDE-100 > PBDE-154, 138. PBDE-17,28,66,71 were very low or undetectable. PBDE-209 was detected in all dust samples and exceeded 300 ug/g at one E-waste site. The most notable differences in the PBDE profiles from E-waste vs. non-E-waste dust was associated with PBDE-209 and PBDE-153, which were 15 - 1000 times higher at E-waste sites. Supported by NIOSH Grant 1R21OH010259-01A1

1698 Internal Exposure to PAHs Using the INTEGRA Integrated Exposure Modelling Paradigm

D. Sarigiannis[1,2,3], S. Karakitsios[1,2] and A. Gotti[1]. *[1]Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Chemical Process and Energy Resources Institute, Centre for Research and Technology Hellas, Thessaloniki, Greece and [3]Environmental Health Engineering, Institute for Advanced Study, Pavia, Italy.* Sponsor: T. Hartung.

This study demonstrates the use of the INTEGRA platform (coupling multi-source multi-route external exposure modeling with a generic PBTK for internal exposure modeling) for estimating exposure to PAHs under a variety of scenarios of key relevance to REACH and the petroleum industry. Internal exposure to PAHs (i.e. arising from all sources a consumer is exposed to: environment, food, consumer products) and the relative contribution from different routes (oral-diet; oral-dust, inhalation, dermal) were calculated. Soil and dust ingestion are the dominant exposure pathways for children (uptake rate of 8.5E-03 ng/kg bw/day, followed by diet (uptake rate for both adults and children at 6-7E-04 ng/kg bw/day) and inhalation (uptake rate at 5.7-9.6E-07 ng/kg bw/day). Oral is the dominant route of exposure (uptake rate at 3.2E-03-15.2E-03 ng/kg bw/day) followed by inhalation (uptake rate at 1E-06 ng/kg bw/day) and dermal exposure (uptake rate at 9E-08 ng/kg bw/day). Internal exposure to PAHs arising from specific use(s)/source(s) was calculated and split up according to uses and sources. More specifically: 1. The contribution of smoking fish to the total PAH content in fish, and consequently the contribution of the consumption of smoked fish to integrated PAH exposure (for fish consumers) is 1.4 ng/kg bw/day whereas the corresponding intake rate from soil or dust ingestion are 0.8-2.1E-03 ng/kg bw/day and from inhalation 4E-07 ng/kg bw/day. 2. The contribution of petroleum products (e.g. RAE - Residual Aromatic Extracts/ OLBO - other lubricants based oils) to integrated PAH exposure

in Europe was reckoned on the basis of: (a) contribution via release of PAHs to the environment (production and downstream use sites of a category of petroleum products) leading to indirect human exposure in the vicinity of an industrial plant of petroleum products. In this case inhalation becomes the dominant exposure pathway (uptake of 2.1E-01 ng/kg bw/day). (b) contribution via consumer use of a category of petroleum products such as lubricants and coatings in rubber boots. Then, the specific consumer use dominates exposure (uptake rate of 6 ng/kg bw/day). In this case, dermal uptake is the dominant route of exposure followed by oral ingestion.

S 1699a Health and Environmental Hazard Assessments of Nanomaterials Along Their Lifecycle

A. Erdely[1] and J. Bonner[2]. [1]*NIOSH, Morgantown, WV and* [2]*North Carolina State University, Raleigh, NC.*

Unprecedented global investment in innovative nanoscale science and engineering has led to the production and utilization of novel materials in expanding fields of electronics, medicine, and composites. Incorporation of advanced materials into existing products through functionalization reactions improves performance, durability, and efficiency in various consumer markets. However, health and environmental hazards of these critical nanomaterials during production, distribution, formulation, use, and disposal have raised concerns. To date, most toxicology research has focused on the as-produced nanomaterial while neglecting the potential health and environmental risks of downstream formulations and applications. This session will highlight 1) exposure potential during production, use, and disposal; 2) hazard identification along the lifecycle of a wide range of nanomaterials; 3) release-testing scenarios with efficacy testing for various nano-enabled products; and 4) the environmental fate of nanomaterials. The outcome of this session will be practical understanding of the most recent research of nanomaterials from a lifecycle perspective. Linking real-world exposures across the lifecycle of nano-enabled products to potential adverse health effects will provide regulators and researchers with essential data needed for effective risk assessment.

S 1699b Rules and Rates of Release From Nano-Enabled Products: Correlating Aging Conditions, the Properties of Product Matrices, and Nanomaterials

W. Wohlleben. *BASF, Ludwigshafen, Germany*. Sponsor: A. Erdely.

Release assessment regards the detachment of a fragment from a larger whole, such as a consumer product during use, and includes the release mechanism, form of the released entity, release scenario, probability of release, and lifecycle simulation, if relevant. Release assessment is already required in specific legislation. Systematic series of release assessment identify key parameters that enable a ranking (and possibly even a grouping) of the probability and characteristics of release from nano-enabled products: The parameter space is spanned by 1. Chemical aging intensity (photolysis, hydrolysis, leaching) 2. Mechanical shear rates (abrasion, drilling, sanding) 3. matrix properties (soft or brittle, resilient or labile) 4. nanomaterial properties (particulate or fibrous, UV-absorbing, -reactive or -transparent). Based on protocol optimization and inter-laboratory validation by MARINA (FP7), nanoGEM (BMBF) projects, the NanoRelease (US, CAN, EU) initiative, we report on quantitative release rates from real-world value-chains: automotive parts, consumer appliances, and wood coatings. Original data from BASF and the SUN (FP7) project shows that release rates are hierarchically determined in decreasing importance by the above ranking 1 to 4: Many release phenomena are process-dominated with important background of nanoscale releases from conventional materials; accordingly, also the physical-chemical and toxicological properties of fragments released from nano-enabled products resemble primarily those of the matrix, modulated by properties of the nanomaterial. Specifically for weathering, the matrix (various polymers, epoxies, cement) determines releases across 5 orders of magnitude from 100 mg release per MJ of UV energy (cements) down to 0.002 mg/MJ (polyethylenes), with less than a factor of 10 up- or down-modulation by the embedded metal-oxide, carbonaceous, or organic nanomaterials. These values can be directly used for mass-flow modeling of nanomaterial emissions.

S 1700 Linking Exposures of Particles Released Across Lifecycle of Nano-Enabled Products to Toxicology: An Integrated Methodology for Particle Sampling, Extraction, Dispersion, and Dosing

P. Demokritou. *Harvard School of Public Health, Boston, MA.* Sponsor: A. Erdely.

Nano-enabled products (NEPs) represent a growing economic global market that integrates nanotechnology into our everyday lives. Increased consumer use and disposal of NEPs at their end of life has led to increased environmental, health and safety (EHS) concerns, due to the potential environmental release of constituent engineered nanomaterials (ENMs) used in the synthesis of NEPs. Although, there is an urgent need to assess particulate matter (PM) release scenarios and potential EHS implications, no current standardized methodologies exist across the exposure- toxicological characterization continuum. Here, an integrated methodology will be presented, that can be used to sample, extract, disperse and estimate relevant dose of life cycle released PM (LCPM), for *in vitro* and *in vivo* toxicological studies. The proposed methodology was utilized to evaluate two "real world" LCPM systems: 1) Particles emitted from nano-enabled toners used in laser printers (PEPs); 2) particles released during incineration/thermal decomposition of nano enabled thermoplastics. This comprehensive framework provides a standardized methodology to assess the release and toxicological implications of ENMs released across the life cycle of NEPs and will help in addressing important knowledge gaps in the field of nanotoxicology. More importantly, the LCPM case studies to be presented will provide 1) toxicological evidence for possible deleterious effects of PEPs released during consumer use (printing); 2) shade light on EHS implications at the end of life of NEPs during incineration.

S 1701 Toxicological Evaluation of Carbon Nanotubes from a Lifecycle Perspective

A. Erdely[1], L. Bishop[1], L. Sargent[1], and J. Bonner[2]. [1]*NIOSH, Morgantown, WV and* [2]*North Carolina State University, Raleigh, NC.*

In recent years, the unique characteristics of multi-walled carbon nanotubes (MWCNT) have been harnessed to advance material science applications. Unfortunately, those same characteristics may also confer adverse health effects. Companies are actively pursuing avenues which apply a surface coating (e.g. polymer or metals) to as-produced multi-walled carbon nanotubes (MWCNT) in an attempt to enhance functionality, conserve material by facilitating dispersion, and decrease human exposure. Currently, very little is known about the toxicity of these coated materials. MWCNT-enabled products, such as composites, once formed, may then undergo sanding or grinding operations, creating a particulate aerosol of MWCNT and composite matrix with the potential for inhalation exposure. As the process moves from the as-produced MWCNT to manipulation of formed composites, the number of potentially exposed individuals increases, although significant reductions in exposure are predicted from nano-release studies and exposure assessments. Toxicologic and pathologic evaluations showed that in several cases, including aluminum or a proprietary polymer coating, pulmonary inflammation and cytotoxicity were reduced after *in vivo* exposure. In addition, pulmonary fibrosis was significantly reduced as a result of surface coating. *In vitro* genotoxicity screening for micronuclei using a pancentromeric probe to detect loss of whole chromosomes and/or chromosome breakage demonstrated that some industrial coatings reduced the cytotoxicity and genotoxicity of multi-walled carbon nanotubes. These findings enable an efficient and accurate safety profiling of MWCNT of varying characteristics along the lifecycle in conditions representing the greatest risk of inhalation exposure and enhance regulatory decision-making regarding the impact of human exposures.

S 1702 Quantifying Exposures from Nanotized Products While Assessing Product Efficacy Across Their Value Chain

P. Westerhoff. *Arizona State University, Tempe, AZ.* Sponsor: A. Erdely.

Past and ongoing research shows that enabling products with nanomaterials (NMs) allows less use of organic chemicals or other potential pollutants, because of the improved performance delivered by the NMs. We have developed multiple release testing scenarios that allow us to assess both release of nanomaterials and permits efficacy testing of the consumer product. Examples from two product lines will be discussed: 1) fabrics treated with CNTs for flame retardancy or Ag0 for antimicro-

bial protection, and 2) food containing TiO2 or SiO2 for color or texture or sunscreen with TiO2. Quantitative data on nanomaterial mass balances during synthesis of the nanomaterials, production of NM-enabled products, release during simulated product use, and transformations through end of life with exposure scenarios to workers, consumers and the environment, and comparisons of nano-enabled products against organic-chemical treated products will be presented. For example, whereas thousands of food products may contain nano SiO2, we benefited by moving up the value chain to procure pure food-grade SiO2 stocks. The stocks were all amorphous nanomaterials with primary particle size in the range of 9–26 nm and demonstrated high consistency in size, morphology, and composition allowing identification and confirmation that 10 out of 14 food products contained amorphous nano SiO2. The dissolution potential study of food-grade SiO2 showed that as high as 6.8% (by weight) of dissolution can occur while un-dissolved SiO2 maintained the same morphology as the food-grade SiO2 stocks in the complex food matrices. This knowledge enabled relevant biological testing on the food-grade SiO2 that showed adverse impacts in the digestive tract (i.e., microvilli) at 1 mg SiO2/L. The association of nano SiO2 with microvilli was not a unique biological interaction as we also observed accumulation of amorphous nano SiO2 on bioflocs in tests using lab-cultured activated sludge and sewage sludge from a wastewater treatment plant. Despite the reputation of food-grade nano SiO2 as being chemically inert and biologically benign, we provide contrary evidence. The project takes a lifecycle perspective from NM synthesis, through product manufacturing, through use phases and end of life, allowing us to understand the efficacy benefits and human/ecological risk tradeoffs of nanotizing consumer products.

S 1703 Environmental Interactions of Nanomaterials in Aquatic Ecosystems

M. Wiesner. *Duke University, Durham, NC.* Sponsor: A. Erdely.

Nanoscale materials are ubiquitous in nature. Engineered nanomaterials and the analytical tools of nanoscience provide have facilitated the study of the transport, interactions and, in some cases, impacts, of nanomaterials on natural systems. This work will summarize a series of laboratory and mesocosm studies designed to evaluate such interactions. Cases of bioüptake, trophic transfer, material transfer and cycling of nanomaterials observed in these experiments will be presented in concert with analysis of the relative toxicity of engineered nanomaterials observed to date compared with more conventional contaminants. Principles for conducting studies using nano-scale phases in toxicity studies will be discussed in the context of transport and transformations of nanomaterials that must be considered.

S 1704 The Promise and Reality of Alternative Methods in Inhalation Toxicology and the Development of Inhaled Therapeutics

J. A. Hotchkiss[1] and A. J. Clippinger[2]. [1]*Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI and* [2]*PETA International Science Consortium, Ltd., Norfolk, VA.*

The respiratory tract is the portal of entry for inhaled gases, vapors, and aerosols into the body. The risk and impact of occupational and environmental exposures to inhaled xenobiotics on human health is often based on guideline-driven inhalation toxicity testing in animals. Similarly, testing the efficacy, pharmacokinetics, and toxicological profile of inhaled therapeutics and excipients is still often conducted in animals. The unique characteristics of the respiratory tract that make it both a potential target organ and a route of systemic exposure present a unique opportunity to develop alternatives to animal tests that are cheaper, faster, based on human mechanisms of action, and that adhere to the 3Rs of reducing, replacing, and refining animal use. Unfortunately, the development, validation, and regulatory acceptance of alternative testing methods for inhaled materials lags behind other exposure routes and organ systems. Much of this may be attributed to the complex interplay of the physical-chemical characteristics of test substances and the regional variation in cell types present along the upper and lower respiratory tract, which together determine the dosimetry, absorption, and metabolism of inhaled materials. This symposium will highlight *in silico* and *in vitro* approaches, including the development and dosimetrically relevant exposure of organotypic air liquid interface (ALI) cultures of human airway epithelial, stromal, and effector cells and application of organ on a chip microdevices to rapidly and efficiently examine exposure-response relationships without the need for traditional *in vivo* inhalation exposure of laboratory animals. The goal of this symposium is to provide participants with a working knowledge of state-of-the-art computational tools and *in vitro* methods currently available and under development to predict acute inhalation toxicity, identify chemical respiratory sensitizers, enhance the testing of inhaled therapeutics and excipients, replace subchronic inhalation toxicity testing, and examine systemic effects and pharmacokinetic properties of inhaled materials.

S 1705 Integrating Alternative Methods within a Regulatory Framework and the Impact of AOPs in Designing an Integrated Testing Strategy to Replace Repeat Exposure Inhalation Toxicity Testing

D. G. Allen. *Integrated Laboratory Systems, Inc., Research Triangle Park, NC.*

Inhalation is a primary route of exposure for gaseous, aerosolized, and vaporized occupational or environmental toxicants. Due to the substantive time, cost, and animal numbers required to conduct traditional *in vivo* toxicity tests, there is much interest in developing cost- and time-effective strategies to assess the toxicity of these materials that are less reliant on the use of animals. This talk will explore the current regulatory landscape associated with required testing for inhalation toxicity within the US and internationally. The hazard information provided by the currently accepted test methods and the associated endpoints measured will be described in order to define the reference against which non-animal alternatives will be compared. A brief overview will be provided on efforts to date that have targeted the development of non-animal approaches to inhalation toxicity, and the data gaps that have been identified as precluding their implementation. The talk will then focus on how non-animal alternatives could be incorporated within the current regulatory framework and identify potential opportunities for collaborative efforts to facilitate progress and implementation. Particular emphasis will be given to the use of adverse outcome pathways associated with exposure to inhaled toxicants to design integrated approaches that combine available *in vitro* and *in silico* methods to identify potential hazards.

S 1706 *In Silico* Models to Predict Acute Inhalation Toxicity and Identify Potential Chemical Respiratory Sensitizers

D. M. Wilson. *Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.*

Currently, there are no approved non-animal methods to predict acute toxicity by any route, including via inhalation. This session will review the state of the science for *in silico* approaches to predict acute lethality via the inhalation route. An overview of our work will be provided with an emphasis on current approaches for read-across. This will include (1) development of an acute toxicity data base that includes data from multiple routes, (2) models that simulate absorption by those routes to predict systemic bioavailability, (3) state-of-the science for addressing metabolism, (4) approaches to distinguish and bin point-of-contact driven effects versus those due to specific mechanisms, and (5) potential use of the OECD Toolbox to profile compounds for their reactivity and potential for respiratory sensitization.

S 1707 Air-Liquid Interface (ALI) Culture Systems and Exposure Modalities As Replacements for Inhalation Toxicity Testing

S. Constant. *Epithelix, Geneva, Switzerland.*

The main function of the human airway epithelium is to generate sterile atmosphere in the alveolar region where the gas exchange occurs. As the first line of defence against airborne pathogens, the airway epithelium acts as key barrier through mucociliary clearance and innate immune defence mechanisms. Airway epithelium is also an important immuno-regulator through production of key messengers and physical interactions with immune cells. Upon activation, respiratory epithelial cells react by producing pro-inflammatory cytokines, chemokines and metalloproteinases to recruit and activate immune cells such as neutrophils, basophils, or to initiate the adaptive immunity via dendritic cells. Interest in the use of 3D reconstituted human *in vitro* tissues (ALI cultures) is increasing in recent years for the study of respiratory diseases such as Asthma, Chronic Obstructive Pulmonary Disease (COPD), Bacterial and viral infections, etc. Genetic and epigenetic diversity among ALIs prepared from single donors allow stratification and patient specific-profiling in toxicology and drug testing. On the other hand, ALIs generated with a mixture of cells from several individuals give a snapshot of global reaction of a small population when exposed to a chemi-

cal compound. Several applications of *in vitro* reconstituted ALI models in inhalation toxicity assessment will be discussed including: (i) acute, long-term and chronic testing strategies utilizing static or dynamic exposure modalities; (ii) inflammatory effect assessment; (iii) trans-epithelial permeability of xenobiotics and (iv) recent advances in detection of respiratory sensitizers and irritants.

S 1708 Co-Culture Systems Mimicking the Human Lung Barrier to Assess the Risk of Inhaled Nanomaterials

B. Rothen-Rutishauser. *Adolphe Merkle Institute, University of Fribourg, Fribourg, Switzerland.*

The lung is considered to be the most important portal of entry for nanomaterials into the human body. For the protection of its large and vulnerable surface the lung is populated with a variety of different types of cells such as pulmonary epithelial cells, macrophages and dendritic cells which are key players in shaping the innate and adaptive immune response. Numerous *in vivo* studies have shown that inhaled nanomaterials deposited on the lung surface can be translocated, either via the blood stream into secondary organs, or by being taken up by immune cells that then migrate into the draining lymph nodes. Although many animal studies are used, increasingly the investigation of potential adverse effects of inhaled nanomaterials are performed using lung cell cultures *in vitro*, thus strongly considering the attempts to reduce, refine and replace (3R) animal experimentations with *in vitro* approaches. A 3D cell co-culture model of the human epithelial tissue barrier composed of epithelial cells, and two antigen-presenting cells, i.e. macrophages and dendritic cells, has been established. The cells can be grown at the air-liquid interface and, in combination with an exposure system that allows investigating nanomaterial-cell interactions at the air-liquid interface, we have a state-of-the-art system to study effects of inhalable materials, their uptake into specific cell types, as well as their translocation rate. With this approach the possible risks of spherical nanoparticles such as gold or silver, but also high aspect ratio nanomaterials, i.e. cellulose whiskers or carbon nanotubes, have been assessed. The advantage of this system is that we can fully control the material characteristics and also monitor the mass deposition on the lung cell surface online, which will allow us to produce a dose-effect correlation. No adverse effects, i.e. oxidative stress or pro-inflammation, for all tested materials mimicking an acute occupational exposure was observed, however, long-term studies resembling a life-time exposure are currently ongoing and also other endpoints such as pro-fibrotic signals will be assessed. We anticipate that such an approach with combining sophisticated 3D lung models and air-liquid exposure will be central to the risk assessment of nanomaterials as well as to the development of novel biomedical nanocarriers.

S 1709 Microengineered Physiological Biomimicry: Human Organ-on-Chips

D. Huh. *Dept of Bio., Department of Bioengineering, University of Pennsylvania, Philadelphia, PA.* Sponsor: J.A. Hotchkiss.

A major problem slowing the development and regulatory approval of new and safer medical products is the lack of experimental *in vitro* model systems that can replace costly and time-consuming animal studies by predicting drug efficacy and toxicity in humans. Here we describe microengineered devices that enable culture of living human lung cells in physiological microenvironments. In particular, this talk will focus on a biomimetic microsystem that reconstitutes the critical functional alveolar-capillary interface of the human lung and exposes it to cyclic mechanical strain and fluid dynamic forces that mimic breathing and blood flow. This microdevice reproduces complex integrated organ-level responses to bacteria and inflammatory cytokines introduced into the alveolar space by inducing expression of intercellular adhesion molecule-1 (ICAM-1) on the microvascular endothelium surface, adhesion of circulating blood-borne neutrophils, their transmigration across the capillary-alveolar interface, and phagocytosis of the infectious pathogens, which can be visualized using real-time, high-resolution microscopy. Using this approach, we developed novel nanotoxicology models and revealed that physiological cyclic mechanical strain greatly accentuates toxic and inflammatory responses of the lung to silica nanoparticles by promoting rapid release of reactive oxygen species by alveolar epithelial cells and upregulating endothelial ICAM-1 expression. Mechanical strain also enhances nanoparticle uptake by the epithelial cells and stimulates their transport into the underlying microvasculature. Importantly, similar effects of physiological breathing on nanoparticle absorption were observed in whole lung using a mouse lung ventilation-perfusion model. Furthermore, we explored the potential use of this microsystem for the development of microengineered models of human lung disease such as pulmonary edema for use in drug screening. This talk will also introduce microengineered airway models and their applications in the study of pulmonary fibrosis. These biomimetic microsystems represent valuable new models for *in vitro* analysis of various physiological functions and disease processes, and provide low-cost alternatives to animal and clinical studies for drug screening and toxicology applications.

S 1710 Concluding Remarks

A. J. Clippinger. *PETA International Science Consortium, Ltd., Norfolk, VA.*

The speaker will summarize the opportunities and challenges for replacement of current *in vivo* test methods for evaluation of inhaled chemicals and therapeutics. Remarks will include a summary of what *in vitro* strategies can be implemented now and what knowledge or technology gaps remain before a reliable replacement for *in vivo* inhalation exposure studies is accepted.

W 1711 Moving Beyond Prioritization towards True *In Vitro*-Based Safety Assessment

M. Yoon[1] and R. Clewell[2]. [1]*ScitoVation, Research Triangle Park, NC and* [2]*ScitoVation, Research Triangle Park, NC.*

The field of toxicity testing is undergoing a global paradigm shift toward the use of *in vitro* approaches for assessing chemical risk. There are already several global initiatives demonstrating the utility of high-throughput screening to prioritize compounds for further testing. The next step for *in vitro*-based toxicity testing is to move from prioritization to prediction i.e., replacing animal-based risk assessment strategies with safety assessments based on human biology queried with *in vitro* assays. This session highlights research aimed at supporting this transition, including development of appropriate cellular assays, selection of predictive *in vitro* biomarkers, definition of points of departure (PoD) *in vitro* and development and improvement of *in vitro-in vivo* extrapolation (IVIVE) tools. We also emphasize the current challenges associated with development and acceptance of these new methods. The first talk describes the need for an integrated testing approach when using *in vitro*-based point of departure with a focus on the development of *in vitro* metabolism systems and biokinetic models that allow dose extrapolation between *in vitro* and *in vivo* scenarios. We will also discuss the use of advanced *in vitro* metabolism systems for *in vitro*-based safety assessment, focusing on challenges for prediction of metabolite-mediated toxicity and tools for *in vitro*-based prediction of repeat exposure toxicity. The topic then transitions to the research efforts to identify biochemical and molecular markers predictive of mode of actions for adverse responses *in vivo*. This talk emphasizes the use of high-content -omic technologies for *in vitro* testing and determining *in vitro* PoDs. The next talk presents a case study of *in vitro*-only safety assessment for use of quercetin in skin lotions. This presentation describes the process of conducting a pathway-based safety assessment in the context of making product safety decisions. The case study demonstrates the process of developing fit-for-purpose *in vitro* assays, setting *in vitro* PoDs, and using IVIVE to translate that PoD to a safe human exposure. The final talk discusses challenges in applying *in vitro* assays for human safety prediction, focusing on approaches in the European Union to increase confidence and reliability of *in vitro* assays for *in vitro*-based safety assessments. Overall, the session provides an opportunity to learn more about the current efforts to merge multiple data streams in order to move *in vitro* testing tools beyond prioritization and create new *in vitro* -only safety assessment approaches.

W 1712 Addressing Metabolism and Kinetics *In Vitro*–Not Just for Dose Extrapolation

M. Yoon. *ScitoVation, Research Triangle Park, NC.*

Consideration of metabolism and kinetics is critical for using *in vitro* data for safety assessments because estimates of safe human exposure conditions depend on the ability to extrapolate from *in vitro* conditions to comparable in-life exposures. To this end, we are developing the advanced *in vitro* metabolism systems that can be used stand-alone or as part of multi-tissue culture system. In this talk, we will present the research progress in the development of long term hepatocyte culture devices, i.e., liver bioreactor, using alginate encapsulated primary hepatocytes cultured in flow-based 3D devices. A few examples of single and repeated dosing studies with selected case compounds will be presented with a focus on the promises of liver bioreactor in generating *in vivo*-like metabolites in large quantities. Computational modeling to support designing the 3D flow based bioreactor and the connected multi-tissue toxicity testing assays will also be discussed. Our research

showed that these advanced *in vitro* metabolism systems will contribute to not only providing accurate estimates of human clearances *in vivo* for dose extrapolation, but also addressing metabolite-mediated toxicity and repeat exposure toxicity *in vitro* by providing realistic exposure profiles in *in vitro* cell-based systems, the major obstacles in moving forward with *in vitro*-based prediction of human safety.

W 1713 Linking Adaptation to Adversity in Human *In Vitro* Experiments to Move Beyond Hazard Identification

P. Jennings. *Medical University of Innsbruck, Innsbruck, Austria.* Sponsor: R. Clewell.

Cells are equipped with numerous pathways to redress perturbations in cellular homeostasis, including chemical induced cell stress. The vast majority of these pathways initiate these responses through transcription factor activation. These include Nrf2 (oxidative stress), HIF1alpha (hypoxia), ATF4 (ER stress), p53 (DNA damage) and MTF (metal stress). It is now possible to unequivocally identify the activity states of the pathways with transcriptomics and other complementary omic assays. This is extremely helpful for hazard identification as we can identify the chemical induced perturbation. However, parenchymal cells particularly in the liver and kidney are very robust and the activation of stress responses alone is not necessarily an indication of adversity. While, the strength and persistence of the adaptive process might be helpful, we have shown in human renal proximal epithelial cells that the activation of stress response pathways is often accompanied by a decrease in differentiation markers (e.g. genes and proteins involved in paracellular permeability, mitochondrial function and transcellular solute transport). We interpret this as a shift of focus from high differentiated function to stress management. Clinically speaking this is an adverse event, e.g. in the kidney, a severe decrease in proximal tubule reabsorption causes Fanconi Syndrome. In addition to differentiation markers, activation of inflammatory responses and cellular repair mechanisms can be indicative of trouble ahead. We will discuss these topics with examples from our research and provide examples of where some of these markers could be translated to clinical settings.

W 1714 Integrating Biokinetics and Biodynamics for Consumer Safety Assessment Using Quercetin As a Case Study

Y. Adeleye. *Unilever, Safety and Environmental Assurance Centre, Bedfordshire, United Kingdom.* Sponsor: R. Clewell.

Perturbation of toxicity pathways has been proposed as an approach for toxicity testing that could ultimately negate the requirement to generate hazard data in animals. Using quercetin (a flavonoid which present in green tea and a variety of berries and vegetables) as a case study, a hypothetical exposure scenario (1% in a skin lotion) and the p53 network as a toxicity pathway for DNA damage, we investigated the applicability of pathway based risk assessment for consumer products. We used *in vitro* assays to determine the Biological Pathway Altering Dose (BPAD) i.e. the biodynamics of the dose significantly altering the pathway that leads to an adverse event. We determined BPADs for quercetin by measuring micronuclei (MN), DNA repair centre formation and transcriptomics in HT1080 cells. We estimated the free concentration of quercetin in *in vitro* assays using both modelling and experimental approaches to convert the BPAD (a nominal concentration) to a biologically relevant dose metric. Finally we used the adjusted BPADs as inputs to a PBPK model, using reverse dosimetry, to estimate a safe inclusion level of quercetin in a formulation. By integrating biodynamics and biokinetics, we demonstrate the practical applicability of using pathway based risk assessment for consumer safety, highlight areas of uncertainty and discuss research needs for making safety decisions based on *in vitro* data.

W 1715 Increasing Confidence and Reliability of *In Vitro* Assays for *In Vitro*-Based Safety Assessments

S. Coecke. *JRC Institute for Health and Consumer Protection, European Commission, Ispra, Italy.* Sponsor: R. Clewell.

In vitro methods, often based on the use of human cells and tissues, are submitted to international validation bodies. Well-designed, robust, reliable *in vitro* methods that can run in a routine environment for generating data sets are becoming more and more instrumental for supporting regulatory decisions. Good *In Vitro* Method Practice (GIVIMP) is a proposal from EURL ECVAM on an international Guidance document on good *in vitro* method practices (GIVIMP) for the development and implementation of *in vitro* methods for regulatory use in human safety assessment, which was recently accepted at the OECD Test Guideline work programme. The major goal of these efforts is towards reducing the uncertainties in *in vitro* based predictions and therefore increasing the acceptance of the *in vitro* estimated safety measures by regulatory agencies. The draft GIVIMP guidance gathers expert input from regulators from European and international receiving authorities such as the European Food Safety Authority, the European Medicine Agency, the European Chemicals Agency, the US Food and Drug Administration, from the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL), from ECVAM's Stakeholder Forum, from the EU and OECD Work Group on Good laboratory Practices, from European 3Rs Centres, from EURL ECVAM's Preliminary Assessment of Regulatory Relevance (PARERE) network, from scientists from large industries and small and medium enterprises and from international scientists with expertise in kinetics, stem cells, cell biology, GLP and *in vitro* methods. The purpose of the GIVIMP guidance is, taking into account all necessary good scientific, technical and quality practices, to ensure that the overall process from *in vitro* method development to *in vitro* method implementation for regulatory use becomes more efficient and effective. An experimental project will be presented as a first GIVIMP-based pilot project aiming at harmonizing and standardizing human hepatic metabolic clearance methods to increase reliability of this class of *in vitro* assays to use in regulatory decisions.

W 1716a Quantitative Cumulative Risk Assessment: Is It Feasible Today?

G. Brorby[1] and M. Mumtaz[2]. [1]*ToxStrategies, Inc., Richmond, CA and* [2]*Agency for Toxic Substances Disease Registry, Atlanta, GA.*

Environmental exposures are complex and include a variety of stressors. Toxicologists and risk assessors continue to be greatly interested in assessing the cumulative risk associated with multiple chemical exposures (such as metals, solvents, pesticides, and persistent organic pollutants) and non-chemical stressors (e.g., stress, noise, biological agents, and socioeconomic status), particularly in underserved, environmental justice communities. Limited studies have been published that document joint exposures to some chemical and non-chemical stressors. Moreover, much of the work conducted to date is qualitative in nature, and methods for quantitatively accounting for the interaction between chemical and non-chemical stressors remain elusive. The workshop will focus on recent efforts to move beyond purely qualitative approaches to cumulative risk assessment, including leveraging existing chemical mixtures risk assessment methodologies, semi-quantitative screening tools, and case studies of unique modeling strategies for quantitatively combining risks from chemical and non-chemical stressors. Limitations of these approaches, and areas for future research, will also be discussed.

W 1716b Can Chemical Mixtures Risk Assessment Methods Guide Development of Quantitative Cumulative Risk Assessment Methods?

G. Rice. *US EPA, Cincinnati, OH.* Sponsor: G. Brorby.

Expert scientific panels have encouraged expanding the focus of human health risk assessment, moving from analyses of risks posed by individual environmental stressors toward cumulative risk assessments (CRAs), i.e., more comprehensive investigations that examine health outcomes associated with the combined effects of multiple chemical and non-chemical stressors. The types of stressors envisioned for consideration in CRAs include harmful biological agents, physical entities such as temperature, and psychosocial stressors, in addition to hazardous environmental chemicals; CRAs also could include other types of stressors such as mutable and immutable factors, diseases and physiological states. The methods that have been used to assess hazards or risks associated with exposures to mixtures of chemicals generally fall into one of the following two categories: component methods and whole mixture methods. Methods available for whole mixtures depend on whether there is information directly available on the mixture of concern or only on sufficiently similar mixtures. Methods available for component data depend on whether there are interactions data available or whether the components act through a similar mode of action or cause the same health effect and are toxicologically independent; based on the available information, the analyst can choose from different component methods such as the hazard index, toxicity equivalence factors, and response addition. There appears to be parallels between these methods and some screening approaches currently being proposed for CRAs. The objective of this presentation is to provide an overview of risk assessment methods for chemical mixtures, and then compare and contrast these methods to emerging methods for CRAs using toxicity or ep-

idemiology data obtained from studies that examined combinations of stressors. While identifying and characterizing risks posed by combinations of non-chemical and chemical stressors in CRAs poses significant and unique challenges, chemical mixture methods can inform methods for quantitative CRAs.

W 1717 CalEnviroScreen: Current Applications and Future Directions in Cumulative Risk Assessment

L. Zeise. *California EPA, Sacramento, CA.*

CalEnviroScreen is a screening methodology developed by the California Environmental Protection Agency (CalEPA) Office of Environmental Health Hazard Assessment (OEHHA) to help identify California communities disproportionately burdened by multiple sources of pollution. The tool uses data on 12 types of pollution and environmental factors (e.g., ozone and PM2.5 concentrations in air, traffic density) and seven population characteristics and socioeconomic factors (e.g., asthma emergency room visits, poverty) to prioritize toxic, health and environmental impacts for each of the state's 8,000 census tracts. While this semi-quantitative screening tool prioritizes cumulative impacts, it is not an expression of health risk. This presentation will discuss the CalEnviroScreen tool, its uses and limitations, and ideas on quantifying cumulative impacts and risks.

W 1718 Cumulative Risk and Adverse Birth Outcomes: The Gulf Resilience on Women's Health Study

J. Wickliffe. *School of Public Health, Tulane University, New Orleans, LA.*

This study was designed to evaluate the cumulative risk of poor birth outcomes (i.e., low birthweight, pre-term birth) associated with an index of chemical (i.e., cadmium, lead, and mercury) and non-chemical (i.e., adverse childhood experiences and trauma, socioeconomic hardship, natural disaster experience) stressors encountered by women in southeast Louisiana. Environmental toxic metal levels were determined in whole blood samples from female human subjects that were between the ages of 18 and 45. Detailed questionnaires were used to obtain personal information regarding adversity and stress experienced by research participants. Birth outcomes were obtained from abstracting medical records or reporting by research participants. Data were transformed into z-scores and quartiles as indices for evaluating exposure to chemical and non-chemical stressors in a cumulative risk model for birth outcomes. Low birthweight appears to be associated with higher levels of exposure to an integrated index of chemical and non-chemical stressors. Our initial results provide a framework for integrating chemical and non-chemical stressors into a cumulative risk framework for birth outcomes. While promising, more work is needed to validate and improve cumulative risk assessments for birth outcomes.

W 1719 Cumulative Risk Assessment of Urban Air Toxics: A Pilot Study in San Antonio, Texas

M. Suh. *ToxStrategies, Inc., Mission Viejo, CA.*

A pilot study was conducted to test a modeling strategy for quantitatively assessing the community health burden potentially associated with certain air toxics (arsenic, lead, antimony, chloromethane) emitted by two coal-fired power plants (referred to as electric generating units, EGUs) located southeast of San Antonio, Texas, while simultaneously accounting for air concentrations of these air toxics from background sources, air concentrations of select criteria air pollutants (ozone, PM2.5), and several non-chemical stressors representative of demographic and socioeconomic variables (age, gender, ethnicity, poverty, violence). Based on ordinary least squares (OLS) linear regression models, demographic and social indicators were evaluated for potential associations with all cancer and heart disease mortality in 34 census tracts within a 10-mile radius of the EGUs. Generalized additive models (GAMs) were then used to examine potential associations between covariate-adjusted mortality rates and the chemical stressors. Four non-chemical indicators (percent African American, percent female, age, poverty) were significantly associated with all cancer and heart disease mortality (70.6% and 79.1% of the variance, respectively); thus, the mortality rates were adjusted for these covariates. Based on GAMs, EGU-related air toxics were not significantly associated with either health outcome. Arsenic from non-EGU sources was significantly associated with covariate-adjusted all cancer mortality (88.8% of residual variance). Lead from non-EGU sources, PM2.5, and ozone, combined, were significantly associated with covariate-adjusted heart disease mortality (88.1% of residual variance). Despite some limitations, this pilot study shows the utility of GAMs to quantitatively assess community health burden and overall, supports that GAMs can be a useful tool in future cumulative risk assessment efforts.

W 1720 A Blood Pressure Effects-Based Cumulative Risk Assessment in a Low-Income Community

J. Peters. *Boston University School of Public Health, Boston, MA.*
Sponsor: G. Brorby.

There is increasing interest in methods for evaluating the joint health effects of multiple stressors (chemical and non-chemical) on health outcomes, using a combination of modeled exposure patterns and epidemiological evidence. Blood pressure is a candidate outcome that has been related to both chemical and non-chemical factors. We used data from the National Health and Nutrition Examination Survey (NHANES) 1999-2008 and structural equation modeling techniques to simultaneously develop predictive models of chemical and non-chemical stressor exposures and their combined effects on blood pressure. For chemical stressors, we focused on lead, cadmium and polychlorinated biphenyls (PCBs). Models were constructed to allow for linkage with public population datasets related to a low-income community near a hazardous waste site. We simulated geographically resolved multivariate attribute data using information from the 2006–2010 American Community Survey and Behavioral Risk Factor Surveillance System. For each individual in the simulated population, we applied the exposure and epidemiological models to characterize multi-stressor exposures, estimate blood pressure, and determine how changes in stressor exposures would influence blood pressure. We illustrated the approach by estimating potential reductions in blood pressure related to hypothetical reductions in lead, cadmium and/or PCB exposures. Model results indicated small shifts in mean blood pressure but also changes in the percentage of hypertensive individuals. Hypothetical reductions in smoking rates demonstrated the importance of the cumulative risk framework given positive associations with lead and cadmium but inverse associations with PCBs and blood pressure. This research demonstrates the ability to jointly consider chemical and non-chemical exposures and their impact on cardiovascular health in the context of cumulative risk assessment.

W 1721a The Role of the Epigenome in Exposure Effects, Susceptibility, and Public Health

S. D. McCullough[1] and D. C. Dolinoy[2]. *[1]US EPA, Chapel Hill, NC and [2]University of Michigan, Ann Arbor, MI.*

The genome contains the blueprint for the structure and function of cells, tissues, and organs; however, the use of this information is controlled by three different regulatory mechanisms: (1) chromatin modifications, (2) DNA methylation, and (3) noncoding RNAs, collectively referred to as the epigenome. The epigenome is a dynamic framework that is responsive to acute environmental exposures, yet changes to the epigenome can also be stable enough to have chronic and even multi-generational implications. Investigations into the role of the epigenome in toxicology are rapidly evolving as a result of novel applications of epigenomics to toxicological studies that are constantly emerging. Thus it is critical to understand the role of the epigenome in cause and effect relationships among exposures, susceptibility, and health outcomes and how those relationships can be examined in the context of public health. The epigenome may hold the key to understanding mechanisms involved in many toxicological phenomena, including the effects of developmental exposures, trans-generational exposure effects, and interactions between chemical and non-chemical stressors in exposure outcomes. Further, recent publicly available tools for epigenomics analysis provide practical opportunities for a broad range of toxicologists to incorporate the epigenome into their translational research program. One such resource, the NIH Roadmap Epigenomics Project, is an integrative analysis of 111 reference human epigenomes from stem cells and primary tissues that represent organ systems that are frequently involved in disease. Through the integration of innovative tools and techniques, such as the Epigenomics Roadmap and longitudinal cohorts, current translational studies are providing novel approaches to understanding the epigenetic mechanisms underlying exposure effects, susceptibility, and risk modification. The goal of this workshop is to explore the recent applications of epigenomics to cutting-edge translational toxicology research. To achieve this we will bring experts together to discuss the involvement of the epigenome in the origins of exposure-related susceptibility and disease from development through adulthood and across generations. The session will open with an overview of the epigenome as a potential mediator of susceptibility and exposure effects following exposure to chemical and non-chemical stressors. The workshop will then focus on two examples of how envi-

ronmental exposures induce changes in chromatin dynamics and DNA methylation that are associated with adverse health effects and susceptibility to the development of future disease. To complement these presentations the session will then discuss publicly available resources that toxicologists can use to integrate the epigenome into their research programs, as well as principles and examples of the integration of epigenomics into multi-omics (transcriptomics, metabolomics, genomics, etc.) datasets to identify biomarkers and characterize mechanisms of toxicity. Further, this workshop will include a panel discussion on the current and future prospects for epigenomics in toxicology. Following this workshop, attendees will have a better understanding of the role of the epigenome in health and disease, and learn how epigenomics can be integrated into translational toxicological studies.

W 1721b Epigenome: Tool for Assessment of Health Impact of Cumulative Exposure to Chemical and Non-Chemical Stressors

K. Olden. *US EPA, Washington, DC.*

During the past few decades, medical research has advanced dramatically and thus improved the health and well being of people in the US and around the world. The World Health Organization has estimated that more than 25% of death and disease worldwide results from environmental exposures; however, while our understanding of human-environment interactions has increased dramatically it still remains incomplete. More recently, it has become clear that an individual's environment can shape his or her epigenome in either a transient or a durable manner. These imprints may hold the potential as biomarkers of exposure history, indicators of susceptibility to the effects of future exposures, and as guides to identify modifiable risk factors that can be used to ameliorate exposure effects. Innovative scientific and regulatory approaches are needed to assess the health impact of environmental exposures that consist of both chemical and non-chemical stressors under "real-world" conditions. To better understand the relationship between environmental exposures and the epigenome we must combine controlled human exposure, *in vitro* mechanistic, and epidemiological studies. This presentation will open the discussion of how the epigenome can be used as a tool to advance our understanding of the molecular mechanisms underlying environmental exposure effects, how chemical and non-chemical factors contribute to differential exposure responsiveness, and how the integration of this information will contribute to the next generation of risk modeling. Further, this presentation will discuss the application of these principles through the Neighborhood Epigenetics Study, which is being led by the US Environmental Protection Agency. This multi-faceted study is exploring the impact of social, geographical, and environmental stressors on the epigenome and how the resulting epigenomic states influence susceptibility to the effects of pollutant exposure. In summary, understanding the role of the epigenome in exposure effects and susceptibility will have tremendous impacts on public health by providing the field of toxicology with information on causal mechanisms and pathways, measurable biomarkers of exposure and susceptibility, strategies to ameliorate exposure effects, and has the potential to transform risk assessment paradigms.

W 1722 Environmental Epigenomics As a Mechanism in Developmental Reprogramming

C. L. Walker. *Texas A&M University, Houston, TX.*

Programming of the epigenome during development directs cell fate, as well as tissue and organ biogenesis. That this developmental programming can also determine the health of an individual, as well as their risk of disease, is what is referred to as the Developmental Origins of Health and Disease (DOHaD) hypothesis. During developmental programming, "set-points" are being established that will determine how an individual will respond to normal physiological signals across the lifecourse, for example determining muscle mass, production of glucose by the liver, and insulin sensitivity. At the molecular level, evidence is also emerging that "set-points" for levels of gene expression, for example transcription of estrogen-responsive genes in response to hormone, may also be determined during developmental programming. Importantly, we are learning that certain environmental exposures can disrupt developmental programming to reset the epigenetic "rheostat" on these genes. We have found in both the developing male and female reproductive tract, environmental exposures that occur during development can reprogram the epigenome to significantly increase transcription of hormone-responsive genes in response to both male (androgen) and female (estrogen) hormones. In animal models for prostate and uterine cancer, this reprogramming and hyper-responsiveness to hormone is associated with increased development of these hormone-dependent tumors. Global and directed analysis of the changes in the transcriptome and epigenome responsible for this reprogramming have identified the polycomb repressive complex (EZH2) and the COMPASS complex (MLL) as key mediators of developmental reprogramming of the reproductive tract in response to environmental exposures.

W 1723 Environmental Epigenomics–Translation to Large-Scale Human Studies

A. Baccarelli. *Harvard University, Boston, MA.* Sponsor: S. McCullough.

Epigenetics investigates heritable changes in gene expression that occur without changes in DNA sequence. Several epigenetic mechanisms, including DNA methylation and histone modifications, can change genome function under exogenous influence. Results obtained from animal models indicate that *in utero* or early-life environmental exposures produce effects that can be inherited transgenerationally and are accompanied by epigenetic alterations. The search for human equivalents of the epigenetic mechanisms identified in animal models is in progress. I will present evidence from human environmental studies indicating that epigenetic alterations may mediate, or at least help to predict, effects caused by toxic exposures. In these investigations, we have shown that exposures, including air pollution, lead, arsenic, nickel, and PAHs, are associated with altered methylation of human repetitive elements or genes. In recent preliminary studies, we have shown alterations of histone modifications and miRNAs in subjects exposed to metal-rich airborne particles. I will present original data demonstrating that altered DNA methylation in blood and other tissues are associated with disease. On the basis of current evidence, I will propose possible models for the interplay between toxicants and the human epigenome.

W 1724 The NIH Roadmap Epigenomics Program, and How It Can Help You!

L. H. Chadwick. *National Institute of Environmental Health Sciences, Research Triangle Park, NC.* Sponsor: S. McCullough.

Environmental exposure-associated changes in the epigenome have been well reported; however, the mechanisms that lead to these changes and impact that they have on exposure effects are less certain. This presentation will discuss the NIH Roadmap Epigenomics Program as a practical and publicly available tool for toxicologists to integrate epigenomics into their research. The NIH Roadmap Epigenomics Program was established to advance our understanding of the critical role that epigenetic processes play in normal human development, and to investigate how perturbing these processes can lead to disease. One of the major achievements of this program was the development of a public data resource of reference epigenomic maps, representing a wide range of normal human cell and tissue types. These reference maps provide a rich resource for researchers interested in understanding how epigenetic modifications distinguish cell types, as well as providing a reference to help interpret the significance of epigenomic variation associated with disease. This talk will provide an overview of the data available and highlight some of the major findings of the first analysis across the data set. I will discuss some of the potential uses of this, and other related large-scale data sets for environmental health researchers.

W 1725 Integration of Epigenomics Data with Other "-Omics" Datasets in Translational Toxicology Studies

R. Shah. *Sciome, LLC, Research Triangle Park, NC.* Sponsor: S. McCullough.

As epigenomics research gains momentum and technological revolution enables scientists to generate large volumes of "-omics" data, the proliferation of multi-omics datasets, comprised of epigenomic, transcriptomic, metabolomic, and other "-omics", poses new and unique challenges for meaningful data analysis. Despite the increased abundance of multi-omics data, it is not yet widely understood how investigators can use these vast volumes of data to reach clear, concise, and cogent conclusions. As the complexity of technical and experimental approaches escalates, it is increasingly important to develop sound strategies that capitalize on the availability of those multi-omics datasets to generate meaningful mechanistic knowledge and further the field of toxicology. This presentation will focus on how investigators can apply bioinformatics tools to dig deep into multi-omics datasets built from epigenomics data generated through chemical and environmental exposure studies combined with "omics" data from publicly available databases, such as ENCODE and the Roadmap Epigenomics

Project. Specifically, two case studies, one from a dexamethasone exposure study and another from a dietary effects study, will be presented to demonstrate how epigenomics (chromatin immunoprecipitation-sequencing (ChIP-Seq)), transcriptomics (microarrays/RNA-Seq), and other –omics data can be integrated into a multi-omics approach to identify the genomic loci that may be altered in response to chemical and environmental exposures. Further, emphasis will be placed on how the analysis and interpretation of multi-omics datasets can help unveil the mechanisms underlying exposure-associated adverse health effects. Finally, examples will be provided to highlight both the challenges and opportunities in leveraging such large datasets for research and discovery as it relates to translational sciences.

W 1726a Using 21st Century Approaches to Evaluate Endocrine-Active Compounds

M. S. Marty[1] and K. Paul Friedman[2]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Bayer CropScience, Durham, NC.*

New 21st century tools are providing valuable information that can contribute to chemical prioritization for endocrine screening and aid in the identification of early events in the mode of action of these chemicals. This session will examine 21st century approaches to evaluating potential endocrine bioactivity and how these data can be integrated for endocrine screening prioritization, endocrine activity determination, and safety assessments. The first presentation will describe an estrogen receptor (ER) model based on the results of ToxCast high-throughput screening (HTS) *in vitro* assays, its performance with reference chemicals, and its application as an alternative assessment of estrogen bioactivity in the US EPA's Endocrine Disruptor Screening Program (EDSP). The second presentation will examine ToxCast HTS results for three triazole fungicides, and how these data can be integrated with other toxicity and exposure data to enable human health-protective EDSP prioritization. The third presentation will focus on the potential targets for chemical-induced epigenetic changes in endocrine signaling pathways during sensitive windows of development, including the role of micro RNAs in multigenerational toxicity, and relevant regulatory assays with potential for adaptation. The fourth presentation will describe the use of an HTS zebrafish embryo model to identify phenotypic and transcriptomic signatures of selected endocrine-active compounds to develop a predictive endocrine disruptor framework. The final presentation will describe the requirements for fit for purpose assays using ER models in a "top-down adverse outcome pathway" approach (defining key events leading to an apical outcome) or a "bottom-up toxicity pathway" approach (defining key events leading to a loss in cellular homeostasis), along with advantages and disadvantages of each in assessing potential endocrine activity. This session critically bridges development of 21st century tools to discussion of their current applications to pathway-based science and regulatory decisions.

W 1726b Screening Chemicals for Estrogen Bioactivity Using Computational Approaches

P. Browne. *US EPA, Washington, DC.* Sponsor: M.S. Marty.

The US Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program has begun a paradigm shift from animal-based methods to using high-throughput and computational approaches for rapidly screening and testing environmental chemicals for endocrine bioactivity. Results from 18 estrogen receptor (ER) ToxCast *in vitro* screening assays were integrated into a computational model that can discriminate estrogen agonist bioactivity from assay-specific interference and cytotoxicity. ToxCast ER model performance was evaluated for reference chemicals, as well as results of EDSP Tier 1 screening assays in current practice. Model accuracy was 86 to 93% when compared to reference chemicals and predicted results of EDSP Tier 1 guideline and other uterotrophic studies with 84 to 100% accuracy. Performance of the ToxCast ER model demonstrates these high-throughput methods correctly identify active and inactive reference chemicals, provide a measure of relative ER agonist potency, and rapidly identify chemicals with potential endocrine bioactivities for additional screening and testing. EPA is accepting ToxCast ER model data for 1812 chemicals as alternatives for EDSP Tier 1 ER binding, ER transactivation, and uterotrophic assays. The EDSP has adopted similar multi-step, performance-based validation approaches of alternative methods for screening androgen and thyroid bioactivities. Adverse outcome pathways (AOP) are used to map EDSP Tier 1 endpoints for screening and develop predictive models, the performance of which are tested against relatively large sets of reference chemicals for *in vitro* and *in vivo* endocrine bioactivities.
The views expressed are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.

W 1727 Predictive Data-Driven Framework for Endocrine Prioritization: Triazole Fungicide Case Study

K. Paul Friedman. *Bayer CropScience, Durham, NC.*

Use of high-throughput screening (HTS) human health hazard and exposure predictions enables efficient prioritization of chemicals subject to the US EPA Endocrine Disruptor Screening Program (EDSP). However, for chemicals with extensive and available endocrine testing information, such as pesticide active ingredients (PAIs), the prioritization approach should be based on an integrated assessment inclusive of all available data. Evaluation of relevant data for three triazole fungicides, triadimefon, propiconazole, and myclobutanil, is presented as a case study in this analysis to demonstrate an integrated assessment for prioritizing PAIs for further endocrine testing related to mammalian toxicology. The evaluation included HTS results, EDSP Tier 1 testing, and other scientifically-relevant information (OSRI) including standard toxicology guideline studies. Systematic comparison of all the data confirmed concordance between endocrine-related HTS bioactivity and the more biologically-complex information that satisfied the EDSP Tier 1 data requirements. Similar margins (3-5 orders of magnitude) were observed between HTS-predicted bioactivity and exposure values and *in vivo* biological activity and EPA chronic exposure estimates for these products' registered uses. The current risk assessment process for PAIs was protective for all *in vivo* effects observed, endocrine or not. Combined HTS hazard and high throughput exposure predictions (ExpoCast) suggest low endocrine testing priority for human health risk of these triazoles. Further, comparison with the mammalian toxicology database for each molecule indicated that this HTS-based prioritization would have been protective for any potentially endocrine-mediated *in vivo* effects (if present). This example demonstrates an effective, human health-protective roadmap for EDSP evaluation of PAIs via prioritization using HTS, available exposure estimates, and guideline toxicology information.

W 1728 The Role of Epigenetics in Toxicity Responses to Endocrine-Active Compounds

M. Jacobs. *Public Health England, Chilton, United Kingdom.* Sponsor: M.S. Marty.

Epigenetic modulations underlie critical developmental processes and contribute to determining adult phenotype. Alterations to the phenotype, due to exposure to environmental insults during sensitive periods of development, are mediated through alterations in epigenetic programming in affected tissues. This presentation will evaluate the potential role of chemical-induced epigenetic modifications to endocrine signaling pathways during sensitive windows of exposure as a mechanism of endocrine disruption, along with the examination of potential methods for assessing such disruption. Potential targets of disruption along putative adverse outcome pathways associated with the signaling pathways have started to be identified, along with assays that show promise in evaluating the target in a screening and testing program. Using a stem cell differentiation cell model, it is possible to elucidate the potential roles of micro RNAs in multigenerational toxicity, for example reproductive system disease, centred around PPAR alpha, has been identified as the major miRNA mediated network affected by estradiol and dibutyl phthalate. Monitoring such epigenetic marks in response to toxicant exposure may in future provide a valuable tool for predicting adverse outcomes, but a more robust basis for regulatory Test Guideline recommendations is still needed, and in development.

W 1729 Using Zebrafish Phenotypic and Transcriptomic Screening to Define Potential Endocrine Activity

R. Tanguay. *Oregon State University, Corvallis, OR.*

We previously screened ~1060 ToxCast phase I and II chemicals using our high-throughput zebrafish platform. From this data set, we aimed to determine if it was possible to generate a predictive endocrine disruption framework by coupling toxicity response data with whole-genome transcriptomics. Importantly, known endocrine active compounds produce adverse developmental outcomes, but the mechanisms underlying these toxic outcomes are largely unknown. Through mining positive 'hits' in our original dataset, quality filtering, and positive control addition, we selected 26 endocrine active compounds for in-depth study. In order to rigorously anchor gene expression to observable phenotypes, we determined an EC80 for each compound. Embryos were exposed to these empirically determined concentrations at 6 hpf and total RNA was collected at 48 hpf, after which whole-genome transcriptomics was completed using high density ArrayXS Zebrafish microarrays. As an example, exposure to 17-ethinyl estradiol produced 980 misregulated transcripts at this developmental time point. Functional analysis of this gene set implicated pathways involved in cholesterol biosyn-

thesis, AhR signaling, WNT signaling, oxidative stress, cell viability, and others. Importantly, beta-estradiol was the most significantly predicted upstream transcriptional regulator of this gene set, demonstrating the usefulness of this method to successfully predict the endocrine target of interest. The resulting analysis of the entire 26 compounds, their respective gene expression signatures illustrate that endocrine active compounds produce adverse developmental outcomes and disrupt target-specific changes in gene expression.

1730 Development of Fit-for-Purpose Assays: Adverse Outcome Pathway and Toxicity Pathway Approaches to Defining *In Vitro* Assays Sufficient for Safety Assessment

R. Clewell. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Initiatives in North America and the EU are driving a transition from animal testing to *in vitro* assays with human cells. Progress in this transition requires "fit-for-purpose" assays that are consistent with normal biological signaling networks of interest and return robust dose-response information. Design and validation of these assays should be guided by adverse outcome pathway (AOP) and toxicity pathway (TP) concepts that connect biological pathway perturbations to cell and organism adversity. Assay development may proceed via a bottom-up approach (defining key events in a TP that can predict loss of cellular homeostasis), or via a top-down approach (defining key events in an AOP that lead to a particular apical endpoint). First, a top-down AOP approach using the current high-throughput estrogen screening assays and expected apical outcomes is discussed. Second, a bottom-up TP-based approach from our laboratory is described. The process involved development of fit-for-purpose assays for estrogenic signaling in human uterine cells with endpoints that reflect key biological processes in estrogen-mediated proliferation, including feedback between five estrogen receptor isoforms that modulate response to hormone fluctuations. Assay validation considered human clinical and epidemiological data. Advantages to using a top-down AOP approach include: providing a highly curated framework to ensure key events are represented in the suite of assays and availability of a well-defined data set for comparing results. The bottom-up TP approach had the advantage that it was not necessary to develop assays for every apical outcome; it may be sufficient to describe the normal biology of a finite number of pathways. Further, as the TP relies on known biology rather than toxicity data, this approach leverages clinical, molecular biology and epidemiologic data for validation. Current advancements and key challenges in defining and validating fit-for-purpose assays that provide dose-response data sets sufficient for safety assessment will be discussed.

1731 Toxicologic Legacies of Major 21st Century Man-Made/Natural Disasters

M. D. Cohen[1] and M. Yoshida[2]. [1]*NYU School of Medicine/Dept. of Environmental Medicine, Tuxedo, NY and* [2]*Institute of Radiation Emergency Medicine, Hirosaki University (IREM/HU), Hirosaki, Japan.*

Just 16 years into the 21st century, there have been a series of major man-made/natural disasters to befall the world, and each has engendered its own particular toxicologic legacy. In 2015–2016, the anniversaries of the World Trade Center disaster (15-year), the landing of Hurricanes Katrina and Rita (10-year), and the tsunami-induced disaster at the Fukushima Daiichi Nuclear Plant (5-year) will be upon us. Similarly, it will be 8 years since the great California wildfires that lasted four months and consumed >1.5 million acres. Each of these events gave rise to adverse health effects among those affected by the disasters, and many of these pathologies are still (or are becoming increasingly) evident. Over the years, clinical and laboratory-based studies have allowed the basis for many of the documented pulmonary/immuno-toxicologic effects from exposures to toxicants generated in each disaster to become clearer. The purpose of this session is to bring together experts to discuss the pulmonary/immuno-toxicologic impacts on human health from each event; a final talk will provide attendees with information about the new NIH/NIEHS Disaster Research Response (DR2) Project and provide recommendations as to how such disasters like those covered in this session should be evaluated (i.e., what to do before, during, after) to mitigate toxicologic risks to those individuals that might be in potentially affected areas. It is expected that this session will provide attendees with the most up-to-date toxicology-related information on each of these major disasters. Accordingly, this will allow for development of better preparation paradigms to mitigate the risks to human health in the event any of these types of disasters occur again.

1732 The Role of the Aryl Hydrocarbon Receptor and its Diverse Ligands in Oral Squamous Cell Cancer Migration and Tumorigenesis

E. Stanford[1], Z. Wang[1], O. Novikov[1], M. Kukuruzinska[2], M. Bais[2] and D. Sherr[1]. [1]*Environmental Health, Boston University School of Public Health, Boston, MA and* [2]*Molecular and Cell Biology, Boston University School of Dental Medicine, Boston, MA.*

The aryl hydrocarbon receptor (AHR) has been widely studied for its ability to be activated by common environmental chemicals, including dioxins, polychlorinated biphenyls and polycyclic aromatic hydrocarbons, all of which are high priority chemicals on the US Agency for Toxic Substances and Disease Registry list of pollutants of greatest concern to human health. More recently, the role of the AHR in stem cell development, self-renewal, maintenance and differentiation has been studied in many contexts, including tissue-specific embryonic stem cells, hematopoietic stem cells, pluripotent stem cells, neural stem cells, megakaryocyte/erythroid stem cells, and breast cancer stem cells. Many studies have implicated the AHR in oral squamous cell carcinoma (OSCC) initiation, but few have looked at the role of the AHR in cancer progression and metastasis. Multiple endogenous and exogenous ligands were tested to see if they activate the AHR in two aggressive OSCC cell lines, SCC2 and CAL27, affect cell migration, or alter primary tumor growth. Complementing parameters of "stem-ness", including ALDH1 activity, tumorsphere formation, and chemoresistance were assessed to test the hypothesis that the AHR similarly influences oral cancer stem cell (OCSC) development and function. Our data indicate that SCC2 and CAL27 cells have basal AHR activity, which can be modulated, altering OCSC population dynamics, and thereby contributing to cancer recurrence and metastasis. The ability of several exogenous and endogenous AHR ligands, including B(a)P and PM2.5, to up-regulate phenotypic and/or functional markers of OCSCs, strongly suggests the potential for ubiquitous environmental AHR ligands to accelerate progression to lethal cancers. Treatment with AHR antagonists lead to: 1) a significant decrease in the percentage of ALDH1high cells, 2) reduced tumorsphere forming ability, 3) reduced cell migration *in vitro*, 4) decreased tumor formation *in vivo*, and 5) increased sensitivity to a frontline chemotherapeutic. The demonstration that AHR inhibition reduces expression of these functional and phenotypic markers encourages the testing of AHR inhibitors as potential chemotherapeutic alternatives and supports the stricter regulation and testing of environmental AHR ligands.

1733 Ligand-dependent Modulation of the Aryl Hydrocarbon Receptor Mediates Expression of the Neutrophil Chemotactic Gene *Cxcl5* in Primary Mouse Keratinocytes

K. J. Smith[3], J. A. Boyer[3], E. Muku[3], K. Gowda[1], S. G. Amin[1], A. B. Glick[3] and G. H. Perdew[3]. [1]*Department of Pharmacology, Penn State College of Medicine, Hershey, PA;* [2]*Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA and* [3]*Graduate Program in Biochemistry, Microbiology, and Molecular Biology, The Pennsylvania State University, University Park, PA.*

Signaling molecules involved in immune system control must be tightly regulated to ensure an appropriate response to external insults and thereby limit an excessive, detrimental reaction. Chemokine (C-X-C motif) ligand 5 (CXCL5), is an epithelial-derived factor involved in the chemotactic recruitment of neutrophils to areas of injury through interaction with chemokine (C-X-C motif) receptor 2 (CXCR2), and exhibits non-redundant functions with other structurally similar chemokines. We demonstrate that the ligand-activated transcription factor, the aryl hydrocarbon receptor (AHR), regulates interleukin-1 beta (IL-1β)-induced Cxcl5 in primary mouse keratinocytes. Electrophoretic mobility shift assays (EMSA) demonstrate the ability of AHR to bind dioxin response elements (DREs) located in the promoter of Cxcl5. Structurally distinct AHR agonists, including the environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and the ultraviolet (UV)-generated endogenous ligand 6-formylindolo[3,2-b]carbazole (FICZ), are capable of synergistically inducing an increase in Cxcl5 expression. The selective aryl hydrocarbon receptor modulator (SAhRM), SGA360, and the antagonist GNF351 are capable of reducing IL-1β-induced Cxcl5 expression. After UVB irradiation, FICZ treatment of C57BL/6 mouse ears increased Cxcl5 expression *in vivo* when compared to UVB exposure alone. These data point to a model whereby exogenous or endogenous ligands, in combination with inflammatory stimuli, activate the AHR and lead to an increase in chemokine gene expression in the skin. This could lead to an overabundance of neutrophils, causing damage to healthy tissue surrounding the site of inflammation. Pharmacological targeting of the AHR could therefore be useful in decreasing acute inflammatory reactions.

1734 Aryl Hydrocarbon Receptor Regulates the Hematopoietic Niche Interactions in Normal Steady State Hematopoiesis

Z. Unnisa, K. P. Singh, E. C. Hemry and T. A. Gasiewicz. *Environmental Medicine, School of Medicine and Dentistry, University of Rochester, Rochester, NY.* Sponsor: K. Singh.

Aryl Hydrocarbon Receptor (AhR) is a ligand activated transcription factor belonging to the Per-Arnt-Sim (PAS) family of proteins. The AHR is activated in response to xenobiotic ligands, leading to nuclear translocation and regulation of hematopoietic stem cell (HSC) functions including self-renewal, proliferation, quiescence, and differentiation. We hypothesize that AhR impacts HSC functions through its interaction with the bone marrow (BM) niche cells and influencing target genes MEIS1, CXCR4, STRA13, HOXB4 and ANGPT1 that have a direct role in HSC maintenance and function. In our experimental design, we used 8 weeks old AhR null-allele (KO) mice along with B6 control mice. The BM from these animals were harvested and analyzed for proliferation assay, LSK (stem-progenitor) cells, qPCR assays, and BM niche cells. Peripheral blood population was not significantly altered despite the intrinsic changes in the HSC populations. There was an increase in osteoblast cells in the KO BM niche and also a significant increase in mesenchymal cells along with a substantial increase in endothelial population constituting the endosteal BM niche. These cell population interactions lead to increased proliferation of in LSK and reduced LTHSC (Long term Hematopoietic Stem cells) in KO animals suggesting decreased self renewal. Our results indicate that AhR is mediating the proliferation and maintenance of HSCs and progenitor populations in the blood and BM, in part, by influencing the microenvironment in the niche cells from where the critical regulatory signals and transcriptional network operates for the origin of these cells. Thus, AhR has a dramatic influence on highly hierarchical hematopoietic system and loss of AhR leads to alterations in BM niche cells influencing higher proliferation in the LSK fraction of the hematopoietic system. We further predict that AhR is important for increased expansion of HSCs, and AHR provides the cell interaction intrinsic microenvironment signals to orchestrate the balance between quiescence and proliferation of HSCs. (Funded by ES01247, ES07026, and ES04862).

1735 Downstream Mechanisms of Aryl Hydrocarbon Receptor Activation: Inhibitory Effects on Osteogenic Differentiation

C. Yun, A. Kannan, S. Mitchell, D. S. Chun, R. D. Freshman, M. Schallmo, J. Weiner, R. Cook, K. Sonn, S. Bellary, C. Park, J. Yun, W. K. Hsu and E. L. Hsu. *Department of Orthopaedic Surgery, Northwestern University, Chicago, IL.*

Tobacco smoke is associated with increased rates of pseudarthrosis (bony non-union) after spine fusion procedures. Numerous toxic ligands for the Aryl hydrocarbon receptor (Ahr) are present in tobacco smoke, and recent evidence suggests that Ahr activation may inhibit osteogenic differentiation. We showed previously that Ahr activation by dioxin inhibits bone regeneration in a rat posterolateral spine fusion model. The purpose of this study was to elucidate downstream mechanisms of dioxin action, and to identify potential therapeutics to mitigate the deleterious effects of dioxin on bone. Bone marrow stromal cells were isolated from rat femurs and tibiae and cultured under standard or osteogenic conditions. Factors critical to osteogenesis were evaluated after exposure to vehicle control, dioxin, Ahr antagonists, or antagonists + dioxin. Ahr antagonists evaluated were alpha-naphthoflavone (ANF; a synthetic antagonist), resveratrol (Res; a stilbenoid found in grapes and present in red wine), 3,3'-diindolylmethane (DIM; a flavonoid product of cruciferous vegetables), and luteolin (Lut; a flavonoid present in celery, broccoli, and other sources). We found that dioxin significantly inhibits ALP activity, BMSC migratory capacity, and matrix mineralization. Co-treatment with each of the 4 antagonists mitigated the effects of dioxin to varying degrees. Dioxin was also found to inhibit BMSC chemotaxis towards several chemoattractants (BMP-2, CXCL12, IL-8, MIP-3α). Co-treatment with ANF and Res generally rescued the anti-chemotactic effects of dioxin to a greater degree than DIM and Lut. RNA and protein expression studies showed that dioxin down-regulates numerous pro-osteogenic genes in rat BMSC. Western blotting showed that co-treatment with Ahr antagonists prevented dioxin-induced expression changes of COL1 and CXCL12 to varying degrees. Our results suggest that Ahr activation may play a critical role in the adverse effects of cigarette smoke on osteogenic differentiation and bone healing. Ahr antagonists should be investigated as a therapeutic option to combat these inhibitory effects.

1736 Preliminary Characterization of the Interleukin-1 Receptor 1, Tumor Necrosis Factor Receptor 1 and 2 Knockout Rats as a Model for Studying the Mode-of-action of the Hepatotoxic Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin

A. Phadnis-Moghe[4], K. Rivera-Caraballo[3], J. Harrill[2], R. Crawford[4], R. Budinsky[1], J. Thomas[1], J. C. Rowlands[1] and N. Kaminski[4]. [1]*The Dow Chemical Company, Midland, MI;* [2]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC;* [3]*University of Puerto Rico, Humacao, PR and* [4]*Institute for Integrative Toxicology, Michigan State University, East Lansing, MI.*

Chronic administration of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) to rodents results in liver tumors. A mode-of-action has been hypothesized that includes hepatocellular necrosis and inflammation as key events in TCDD-induced liver tumor promotion. It has been reported that the cytokines such as IL-1α and β, TNFα and β act as downstream mediators of rodent liver inflammation, as mice lacking the receptors for these cytokines were refractory to dioxin-induced liver toxicity. Hence, to assess the mode-of-action for TCDD-mediated hepatotoxicity in the rat, a model specifically lacking receptors for IL-1R1, TNFR1 and TNFR2 was generated. Deletions of the receptor genes were confirmed via sequencing and q-PCR using mutation-specific primers in the single and triple knockout (KO) rats. Prior to investigating the effects of TCDD in the KO rats, a systematic characterization of the KO rats was conducted, which involved detailed analysis of the immune cell composition in the spleen and liver of these rats by flow cytometry. Quantitation of gross organ weight to body weight ratio showed a significant decrease in the spleen weight of the IL-1R1 KO and TNFR2 KO rats, possibly due to a significant decrease observed in the percentage of B cells and dendritic cells. The proportion of CD68+ and CD163+ macrophages however, were higher in the TNFR2 KO in comparison with the other KOs. In contrast, percentages of the CD8+ T cells, NKT cells were significantly lower in the TNFR2 KO rats as compared to the WT or other KOs. Taken together, these studies offer an initial characterization of the knockout rat models, suggestive of differential immune cell composition. Experiments are currently underway to analyze the responses of these knockout rats to inflammatory cytokine stimuli. (Supported by The Dow Chemical Co.)

1737 Ah Receptor Expression in Mouse Embryonic Stem Cells Delays Cell Cycle Progression Through Mitosis and Causes Pluripotency Loss

C.-I. Ko, Y. Fan, Q. Wang, M. de Gannes, Y. Xia and A. Puga. *Environmental Health, University of Cincinnati, Cincinnati, OH.* Sponsor: A. Puga.

Oscillating expression of pluripotency factors in embryonic stem (ES) cells creates distinct responses to differentiation stimuli and program ontogeny. Uninterrupted passage through S-phase contributes to pluripotency maintenance by constantly renewing the population of ES cells. The aryl hydrocarbon receptor (AHR) is an environmental sensor that plays a role in regulating cell cycle progression. Our recent studies suggest that AHR repression is required to maintain ES stemness. Here we report the findings that AHR expression in ES cells oscillates between states of high and low levels. A subset of high AHR-expressing (AE) ES cells shows loss of pluripotency and abnormal differentiation pattern with decreased cardiomyocyte contractility. These AE cells proliferate more slowly than the rest of the population and have a shorter S-phase and an increased G2/M-phase. Analysis of mitotic signaling in AE cells show prolonged progression through mitosis, with decreased PP2A activity and phosphorylated-Y15-CDK1 (pY15CDK1) levels, lower 14-3-3 and highly elevated CDC25B protein expression. MID1 expression in AE cells is largely reduced, supporting the conclusion that AHR delays ES cell mitosis by disrupting the MID1-PP2A regulatory circuit. We find the opposite effects in mouse Ahr-/- ES cells, including higher S-phase and lower G2/M-phase cell populations, increased pY15CDK1 levels, higher 14-3-3 and higher MID1 protein expression. Knockdown of the Mid1 gene results in decreased PP2A activity, whereas ectopic overexpression of human AHR (AHR-OE) in Ahr-/- ES cells up-regulates CDC25B expression, and knockdown of the Cdc25b gene in the AHR-OE Ahr+/+ ES cells rescues the ES proliferation rates. Our results suggest the involvement of an AHR-MID1-PP2A-CDC25B axis that regulates ES cell mitosis. We conclude that AHR expression in ES cells disrupts the mitosis with MID1-associated PP2A and CDC25B dysfunction that correlates with pluripotency loss. Supported by NIH Grant 5R01ES06273.

1738 Regulation of Ahr Expression by an Orphan Nuclear Receptor Nr2e3

K. Tilak, D. Kim and K. Kim. *Environmental Health, University of Cincinnati, Cincinnati, OH.*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that binds various environmental toxicants and mediates xenobiotic metabolism by inducing CYP1 family of detoxification enzymes. It has been increasingly evident that AHR plays other important roles in inflammation, metabolic disorder and cancer. However, the underlying mechanism that regulates AHR expression is still largely unknown. Our previous report showed that an orphan nuclear receptor NR2E3 gene network is involved in AHR signaling pathways. To our surprise, we discovered that NR2E3 depletion significantly reduced AHR expression and environmental toxicant-mediated induction of CYP1 family of enzymes whereas NR2E3 overexpression increased AHR expression and responsiveness in human cancer cells. Results from co-immunoprecipitation assay and co-localization study showed that NR2E3 directly interacts with coactivator SRC2. The formation of transcriptionally active NR2E3/SRC2 protein complex and the presence of active histone mark H3K4me2 on AHR gene promoter region were confirmed by ChIP assay. In contrast, NR2E3 depletion recruited LSD1, a histone demethylase for H3K4me2, to the AHR gene promoter, resulting in decreased H3K4me2 mark and AHR expression. The role of NR2E3 *in vivo* was further investigated by employing NR2E3rd7/rd7 mutant mice that express low NR2E3 levels. We consistently observed that AHR expression and the induction of CYP1 family of enzymes by environmental toxicant exposure were markedly decreased in the liver of NR2E3rd7/rd7 mice. The disruption of active transcriptional and epigenetic status on AHR gene promoter was also detected by *in vivo* ChIP assay. Lastly, we examined whether inhibition of LSD1 histone demethylase activity can affect AHR expression *in vivo*; Administering of SP2509, a LSD1 inhibitor, into the liver of NR2E3rd7/rd7 mice fully restored AHR expression. Collectively, our results present a novel regulatory mechanism for AHR expression by establishing NR2E3 as an upstream epigenetic regulator. Results indicate that dysregulation of NR2E3-AHR signaling axis likely plays a role in AHR-associated human pathological processes and also suggests that LSD1 inhibitor can be developed as an epigenetic drug for treating AHR-related human diseases

1739 Understanding the Role of the Integrated Stress Response in iPSC Derived Human Cardiac Myocytes: From Progenitor Cell to Differentiated Cardiac Myocyte

G. H. Searfoss, J. A. Willy and T. K. Baker. *Investigative Toxicology, Eli Lilly and Company, Indianapolis, IN.*

Disruptions of normal cellular protein synthesis/processing, endoplasmic reticulum function, and nutrient starvation can lead to the induction of the integrated stress response (ISR) program in an attempt to preserve and restore cellular function by activation of EIF2 kinases, resulting in phosphorylation of eukaryotic initiation factor 2 on the alpha subunit (eIF2α~P) and resulting activating transcription factor 4 (ATF4). If the stress is unresolved, prolonged activation of the ISR will result in elevated levels of CHOP, and resulting cell death. A cell's ability to respond to these stressors likely depends on the overall differentiation state of the cell. Differentiated cells derived from induced pluripotent stem cells (iPSC) provide a useful model for examining a cells ability to mount an ISR depending on its state of differentiation, as all the cells derive from a single initial iPSC and cells along the differentiation lineage will share the same genetic and epigenetic background. Using iPSC derived human cardiac myocytes and their proximal progenitor cells, we show that the cardiac myocyte progenitor cells display a more robust response to inducers of cell stress with increased levels of ISR stress response mediators ATF4, and CHOP compared to differentiated cardiac myocytes. Consequent with increased activation of the ISR, the progenitor cells are more sensitive to cellular stress and show increased cytolethality relative to fully differentiated cardiac myocytes. These data provide insight into possible differential susceptibility of early progenitor cell pools to integrated stress response inducing treatments *in vivo*, and provides a possible screening tool for identifying molecules that may differentially impact progenitor cell pools.

1740 Evaluation of Acute and Chronic Effects of Kinase Inhibitors on Electrical and Contractile Activity of Human iPSC-Derived Cardiomyocytes

X. Zhang, B. Xi and Y. A. Abassi. *Biology, ACEA Biosciences Inc, San Diego, CA.* Sponsor: C. Jin.

Tyosine kinase inhibitors (TKi) have revolutionized treatment of multiple types of cancer and can induce tumor shrinkage and prolong patient survival. However, they may also have certain degree of on target or off-target cardiovascular side effects in a subset of treated patients. In many cases, existing preclinical safety evaluation of the compound, which mainly focuses on animal toxicity studies and blockade of human Ether-a-go-go related channel (hERG), were not able to anticipate adverse cardiac effects. Therefore, there is an immediate need to develop new predictive preclinical assays and screens to test the potential adverse effects of kinase inhibitors. Here, we report a new *in vitro* screening assay to evaluate potential acute and chronic cardiac liability of TKi (sunitinib,dasatinib, nilotinib, sorafenib, lapatinib, erlotinib and imatinib) . We treated human induced pluripotent stem cell-derived cardiomyocytes (hiPS-C) with a panel of FDA-approved TKis for up to 2 days. Acute and chronic drug effects on attachment, viability, contraction and electrophysiology of iPSC were simultaneously examined and quantified using the xCELLigence RTCA CardioECR system. Tested drugs displayed differential effects acute and chronic effects on integrated ion channel activity as well as contractility. Furthermore, a number of kinase inhibitors which appeared to be safe based on acute affects, displayed longer term chronic effects on hiPSC. Taken together, this multi-parameter assay using hiPSC in conjunction with simultaneous measurement of ion channel activity, contractility and viability can provide incisive information on TKi-induced cardiotoxicity.

1741 Assessment of the Electromechanical Window of Human IPSC-Derived Cardiomyocytes and Its Modulation by Compounds Affecting Cardiac Function

X. Zhang, B. Xi, X. Wang and Y. A. Abassi. *Biology, ACEA Biosciences Inc, San Diego, CA.* Sponsor: C. Jin.

The heart is an electromechanical pump which is regulated by electrical signals emanating from the SA node controlling the extent and timing of cardiac output. The intricate relationship between electrical signals and mechanical output or contraction is also preserved in cardiomyocytes, the individual functional units of the heart. We sought to investigate and characterize the precise timing of the electrical and contractile activity of human iPSC-derived cardiomyocytes and its modulation by reference compounds using the xCELLigence RTCA CardioECR System. The CardioECR system utilizes specialized plates containing both impedance and field potential electrodes. The field potential electrodes measure the integrated ion channel activity of cardiomyocyts while the impedance electrodes simultaneously measure the contractile activity. First, we optimized assay conditions in terms of cell seeding density and culture conditions to obtain stable field potential and impedance signals. Using the field potential and contraction waveforms, we derived a parameter termed Electromechanical Window (EMW) which is the timing difference between integrated ion channel activity and contractile activity. Under normal assay conditions and once electrical and mechanical connections are formed, the EMW becomes stable. Treatment of the cells with compounds which modulate ion channels such as sodium and calcium significantly affects the timing of the field potential duration as well as contractile signals to the same extent which does not significantly affect the EMW. IKr inhibitors such as E4031 also does not affect the EMW at sub EAD doses. However, concentrations which cause EAD can significantly change the EMW. Furthermore, compounds which only affect the contractile machinery of the cardiomyocytes do significantly affect the EMW by either increasing or decreasing the EMW depending on mechanism of action. In summary, simultaneous measurement of electrical and contractile activity of cardiomyocytes and assessment of the timing of these events can provide further insight into mechanism of compounds modulating cardiac function.

1742 High-Throughput Comprehensive Cardiac Safety and Toxicity Assessment Using Human iPSC-Derived Cardiomyocytes

R. Kettenhofen[1], A. Duenbostell[1], O. Keminer[3], G. Luerman[2] and M. Stevens[2]. [1]*Axiogenesis AG, Cologne, Germany;* [2]*Axiogenesis Inc, Plymouth Meeting, PA and* [3]*Fraunhofer IME, Aachen, Germany*. Sponsor: E. Clarke.

A major hurdle to overcome in drug development is drug-induced hERG block leading to QT-prolongation and their association with potentially life threatening ventricular cardiac arrhythmias, a.k.a. Torsade-des-Pointes (TdP). The FDA/HESI/CSRC has sponsored the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative - a new model for a mechanistic approach to cardiac safety testing that improves upon existing methodologies (i.e. hERG testing) to better predict proarrhythmic activity through the use of ion channel screening, computer modeling, and stem cell derived cardiomyocytes. Outside of CiPA and arrhythmia concerns, structural cardiotoxicity or chronic dosing effects on intracellular signaling pathways are still of great concern as these effects can influence lead candidate selection dramatically. Thus, the development of novel cardiotox screening platforms to enhance drug discovery during early preclinical development is of great value to potentially reduce costly downstream attrition. Here we present work with human induced pluripotent stem cell (iPSC) cardiomyocytes, used to compare the effects of dofetilide, E-4031, flecainide, moxifloxacin, ranolazine, quinidine, nifedipine, verapamil, TTX, and ATX II reference compounds in either manual patch clamp and/or microelectrode array (MEA) recordings and HTS compatible 384 well fluorescent calcium and voltage sensitive dye assays. We also present high content imaging results from cardiomyocytes treated with tyrosine and broad spectrum kinase inhibitors, as well as other oncological drugs demonstrating cardiac liability. These data shows the flexibility and predictivity of human iPSC - derived cardiomyocytes as a HTS-scalable tool for comprehensive cardiac risk assessment.

1743 Multimodal Screening of Functional and Structural Drug Toxicities Using Human Stem Cell-Derived Cardiomyocytes

M. Brock, J. Heidmann, A. Kauss and D. Misner. *Safety Assessment, Genentech, South San Francisco, CA.*

Cardiomyocytes derived from human stem cells have revolutionized the practice of *in vitro* cardiotoxicology. In culture, these cells form a syncytial monolayer with highly-regular spontaneous beating lasting weeks to months, and low well-to-well variability in beat properties, enabling functional screening in label-free multiwell assays. We demonstrate up- and downstream coverage of E-C coupling using two platforms. Electrical signals are recorded non-invasively from multiwell plates containing substrate-embedded multielectrode arrays. Field potential waveforms show acute dose-dependent modulation by anonymized internal compounds consistent with identified ion channel liabilities. Contractility is assayed in parallel by automated video analysis of beating in standard multiwell plates. The non-invasive nature of both recording methods permits tracking of "structural" toxicity that develops over days to weeks. For example, we present data from a series of internal "GX" compounds selected for *in vitro* investigation due to reductions in heart-to-brain ratio and ultrastructural signs of cardiomyocyte atrophy in rat studies. While acute effects of GX compounds on cardiomyocyte beat properties are minimal, profound dose-dependent reductions in amplitudes of contraction and field potential spikes develop over hours to days, in the absence of detectable cell death. To multiplex electrical and contractility endpoints for future experiments, we validated two new strategies: 1) A recording system for plates in which field potential is sensed concurrently with membrane impedance, a measurement reflecting both real-time changes in cell morphology with beating, and long-term changes due to drug effects on monolayer integrity. Results for GX compounds confirmed those described above: field potential and contractility decreased in a time- and dose-dependent manner, with little effect on monolayer viability. 2) Transparent 48-well MEA plate prototypes were used for sequential recordings of field potential and video, as well as terminal high-content imaging to assess cell morphology.

1744 Cardiotoxicity of Anticancer Drugs Assessed by Electrophysiology- and Biochemistry-Based Assays Using Human Induced Pluripotent Stem Cell (hiPSC) Derived Cardiomyocytes

T. de Korte, F. Stevenhagen, S. C. den Hartogh, K. Langenberg, M. Dunska, C. Grandela, S. R. Braam and M. L. Vlaming. *Pluriomics B.V., Leiden, Netherlands.* Sponsor: J. Louisse.

Cardiotoxicity is a major cause of attrition during (pre)clinical anticancer drug development [1] and a growing concern with increasing survival of cancer patients [2]. Many anticancer drugs inhibit kinases that are critical for both cancer cell growth and cardiac homeostasis, leading to the destruction of cancer cells as well as cardiac dysfunction. The main cause of failure in early cardiotoxicity screening is the lack of relevant disease models and assay technologies that can mimic the complex conditions of the human cardiovascular system and subsequently detect cardiac side effects. We developed a combination of novel cardiotoxicity assays based on our hiPSC-derived cardiomyocytes (Pluricyte® Cardiomyocytes) to determine cardiotoxicity of (novel) pharmaceuticals. In addition to assays that can detect arrhythmic effects (e.g. multi-electrode array assays), we also set-up other functional assays to study structural toxicity (e.g. through cell viability measurements) and alterations in contractility (e.g. by impedance measurements). For a set of oncology drugs (doxorubicin and the tyrosine kinase inhibitors sunitinib, imatinib, lapatinib and nilotinib) we investigated cardiotoxic effects in Pluricyte® Cardiomyocytes through a range of biochemistry- and electrophysiology-based assays. Addition of these drugs led to the expected alterations in beating patterns and a concentration- and time-dependent reduction in cell viability. The data presented here support the use of hiPSC-derived cardiomyocytes to assess toxic effects of cardio-active compounds in humans, thereby improving preclinical testing strategies for cardiotoxicity. Our platform, which combines state-of-the-art human cardiomyocytes (Pluricyte® Cardiomyocytes) with biochemistry- and electrophysiology-based assays, enables early cardiotoxicity screening while at the same time greatly reducing the use of animal experiments in preclinical development. [1] Khan et al., British Journal of Pharmacology, 2013; [2] Sheppard et al., Frontiers in Pharmacology, 2013

1745 Identification of the Cellular Effects of Sofosbuvir and Amiodarone Using hiPSC-Derived Cardiomyocytes

D. C. Millard[1], C. J. Strock[3], S. Stoelzle-Feix[4], N. Becker[4], K. Juhasz[4,5], N. Fertig[4], C. T. January[6], B. D. Anson[2] and J. D. Ross[1]. [1]*Axion Biosystems, Inc, Atlanta, GA;* [2]*Cellular Dynamics International, a Fujifilm Company, Madison, WI;* [3]*Cyprotex, Watertown, MA;* [4]*Nanion Technologies GmbH, Munich, Germany;* [5]*Technische Universität München, Munich, Germany and* [6]*School of Medicine and Public Health, University of Wisconsin, Madison, WI.*

Sofosbuvir-based drugs have significantly advanced care for hepatitis C virus (HCV)-infected patients. While exhibiting a clean safety profile throughout development, recent post-marketing reports indicate that severe symptomatic bradycardia can occur through combined administration of sofosbuvir and amiodarone. The underlying cellular mechanism of this drug-drug interaction remains unknown. Here, we present a mechanistic interrogation of the cellular effects of sofosbuvir and amiodarone using human stem cell derived cardiomyocytes (hSC-CMs), multiwell microelectrode array (MEA), automated patch clamp (APC), and impedance measurements. MEA recordings demonstrated that sofosbuvir alone had no effect on hSC-CM electrophysiology, but reversed the effects of amiodarone when added in combination, leading to a 53% decrease in field potential duration and 100% increase in spontaneous beat rate relative to vehicle controls. APC assays demonstrated that the drug combination did not block potassium, sodium, or calcium currents beyond the assay controls. Simultaneous MEA and impedance measurements revealed differential responses on electrical and contractile activity; hSC-CMs remained electrically active while contractile activity ceased within ~2 hours of application with the drug combination. The impact on cardiomyocyte electrophysiology and contractility without a direct effect on ion channel activity suggests that the combination of amiodarone and sofosbuvir may impair intracellular calcium handling. These results demonstrate the importance of measuring multiple endpoints within the functional excitation-contraction cascade and, more generally, reinforce the utility of hSC-CMs as an integrated assessment of cardiac safety liability *in vitro*.

1746 Drug-Induced Taste Change in Clinical Practice and Preclinical Safety Evaluation

T. Wang[1] and K. Mansfield[2]. [1]*Novartis Pharmaceuticals, Emeryville, CA and* [2]*Novartis, Cambridge, MA.*

Drug-induced taste disorder is one of the most distressing adverse side effects reported by chemotherapy patients. Taste change (dysgeusia) not only reduces the quality of life for affected patients, but can lead to malnutrition, weight loss, and, in severe cases, difficulty in maintaining a therapeutic regimen. The goal of this session is to review current understanding of taste changes in clinical practice, and how the identification of taste change in preclinical investigations in animal models can provide mechanistic insights and help develop mitigation strategies to guide clinical practice. The symposium will review taste change in patients, including clinical symptoms, type of dysgeusia, diagnostic methods, and current approaches to the treatment of taste disorders. Since smell and taste are closely related, a follow-up presentation will focus on the biochemical and physiological mechanisms of drug-induced changes, and describe enrichment procedures help patients overcome aberrant taste and smell. After presentation of the clinical perspective, the preclinical safety evaluation of taste changes with an emphasis on morphological evaluation and biomarker in rats and dogs will be presented. The description of morphological and molecular tests for taste change will be followed by a comprehensive presentation of physiological methods for functional evaluation of taste change in preclinical animal models. The presentations will also outline how these functional tests can facilitate the development of approaches to overcome dysgeusia.

1747 Taste Disorders in Patients: Symptoms, Diagnosis, and Treatment

M. Grushka. *William Osler Health System, Toronto, Canada.* Sponsor: T. Wang.

Taste changes are both common and complex. They can range from partial to complete taste loss to taste confusion so that that foods no longer taste "right" and are often unpleasant. Equally distressing are the onset of taste phantoms (Dysgeusia) which are often bitter or metallic and can become progressively more intense over the day to the point where they may become unbearable. While loss of taste is common, the cause is often unknown. The current literature suggests that partial loss of taste affecting one nerve, e.g. the chorda tympani, can precipitate a loss of inhibition phenomenon, so that taste carried by the remaining nerves, including the glossopharyngeal, may become spontaneously active in the absence of stimulation, and play a key role in the production of oral sensory taste phantoms. Recent data also suggest that oral pain phantoms can also occur when there is loss of inhibition to the lingual nerve in susceptible individuals following taste loss. Although there are a number of methods for clinical taste testing, this review and assessment will focus on spatial taste testing, which uses suprathreshold tastants to look for loss or alteration of sweet, salty, sour or bitter taste at the tip and back of the tongue to identify areas of taste loss or taste confusion. Clinical smell testing will also be briefly reviewed to investigate the possibility that alteration of taste is linked to partial or complete smell loss resulting in a loss of flavor. This presentation will review the recent literature concerning current treatment options.

1748 Drug-Induced Taste and Smell Disturbance

T. Hummel. *Smell & Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Fetscherstrasse, Dresden, Germany.* Sponsor: T. Wang.

Advances in the understanding of the sense of smell have increased awareness of the role of olfaction in human life. Odors and tastes are, at least in part, perceived via specific G protein-coupled receptors with cAMP or IP3 as the second messenger. Drugs that interact with this signaling cascade, such as opioids, cannabinoids and sildenafil are known to reduce olfactory function. Drugs that are active in the central nervous system may also interfere with the complex processing of olfactory information to distinguish, via pattern recognition, thousands of odors from the signals of only ~400 distinct olfactory receptors. For taste 100s of drugs have been reported to cause taste disorders. However, olfactory or gustatory drug effects have rarely been explored in controlled studies. In our current prospective cross-sectional study 1008 outpatients at a general practitioner were enrolled. All currently taken medications were obtained and olfactory function was assessed using an odor identification test. The association between the patients' sense of smell and the administered medications was based (i) on the active pharmacological substances and (ii) on the molecular targets queried from the publicly accessible DrugBank database. The administration of levothyroxine was associated with a higher olfactory score ($p = 0.033$). For the 168 drugs 323 different targets could be queried. Besides agonistic targeting of thyroid hormone receptors, antagonistically targeting the adrenoceptor alpha 1A was associated with a significantly higher olfactory score ($p = 0.012$). In summary, using state-of-the-art techniques the identified drug effects on olfaction were both biologically plausible based on supportive information from basic science studies.

1749 Preclinical Safety Investigations of Drug-Induced Taste Change in Animal Models–Histology and Biomarkers

K. Mansfield. *Novartis Pharmaceuticals, Cambridge, MA.* Sponsor: T. Wang.

Evaluation of potential drug-induced dysgeusia in animals requires specialized techniques for sample collections, as well as biomarker assessment. The presentation will focus on the preclinical evaluation of the taste change from both morphological and biomarkers aspects. Taste buds are small collections of neuroepithelial cells that reside within the epithelial mucosa of the tongue and contain 50-60 cells of several subtypes. These include type I, type II and type III chemosensory cells as well as type IV cells representing the putative stem cell population. These cell types differ in function and in longevity but rapidly turnover with half-lives calculated to be between 8 days for type II cells and 21 days for type III cells. H&E evaluation of tongue samples collected in the standard toxicological assessment of preclinical species provides limited ability to examine taste bud morphology and cellular composition. However focused sampling of the circumvallate papillae can ensure adequate numbers of taste buds are collected for evaluation. Molecular localization tools can be used to delineate epithelial cells (CK7), taste sensory cells (GNAT3, NCAM/CD56) and taste bud stem cells (LGR5), and the innervation (PGP9.5) to the gustatory system. Molecular localization can be coupled with quantitative image analysis to demonstrate alterations in taste bud cellular composition and growth kinetics. Once learned, these techniques can be readily incorporated into routine preclinical toxicology studies to evaluate potential drug-induced dysgeusia.

1750 Chemosensory Assessment in Animal Models Undergoing Chemotherapy Treatment

J. Glendinning. *Barnard College, Columbia University, New York, NY.* Sponsor: T. Wang.

Chemosensory disorders afflict many cancer patients under chemotherapy. The disorders make foods and beverages taste unpleasant, and thus lead to malnutrition and weight loss. Little is known, however, about the etiology of these drug-induced changes in chemosensory function. This stems in part from the absence of rodent model systems for exploring the molecular, cellular, physiological and perceptual changes. I will discuss strategies for determining whether a particular drug alters chemosensory function, and if so, the nature of the alterations. To help identify the modality of the chemosensory change, there are high-throughput behavioral assays for assessing changes in responsiveness to a battery of chemical stimuli known to selectively stimulate the taste, trigeminal or olfactory system. If these latter tests reveal modality-specific changes (e.g., enhanced responsiveness to low concentrations of salts), there are electrophysiological methods for recording from the relevant chemosensory organs to determine whether the chemosensory changes are mediated (at least in part) in the peripheral sensory system. Such findings would provide a rational basis for designing therapies that either mask or minimize the unpleasant chemosensory sensations.

1751a Genotypic and Intrinsic Risk Factors That Increase Susceptibility to Inhaled Pollutants

D. B. Miller[1] and G. Knudsen[2]. [1]*University of North Carolina-Chapel Hill, Chapel Hill, NC and* [2]*National Cancer Institute at NIEHS, Research Triangle Park, NC.*

The mandated Clean Air Act (CAA) requires the Environmental Protection Agency to set National Ambient Air Quality Standards (NAAQS) to mitigate high-level harmful emissions from natural and man-made sources. These standards strive to protect the health of the most vulnerable human population from potential pollutant-induced adverse health outcomes. However, because of limited research on susceptible subpopulations, the determinations of NAAQS for at-risk groups have proven to be difficult. As a result, an uncertainty factor of 10 is employed in the absence of actual data on susceptibility. A few epidemiological and experimental studies have implicated risk factors such as genotype, sex, ethnicity, life stage, and underlying diseases in human inter-variation

that increase susceptibility to adverse health effects of inhaled pollutants. However, the mechanisms of how these factors increase sensitivity to inhaled pollutants in at-risk groups have not been well understood. The aims of this symposium are to 1) address the underlying health conditions that can influence risk of adverse health effects associated with inhaled pollutants and 2) identify biologically plausible mechanisms and/or interactions between these risk factors and inhaled pollutant injury. The six trainee presenters will focus on different risk factors and examine how underlying conditions alter the adverse health effects of inhaled pollutants, with the emphasis on potential mechanisms. The first two presenters will focus on gene-environment interactions that affect susceptibility to inhaled pollutants, while one of the speakers will also address the differential risk due to sex. The third speaker will use *in utero* data to discuss how air pollution exposure during different life stages can modify health risk. The last three presenters will focus on pre-existing respiratory and metabolic conditions that have been implicated in increased susceptibility to inhaled pollutants. Altogether, attendees will learn about different characteristics that influence susceptibility and gain insight into likely mechanisms by which air pollution effects are altered in at-risk groups. Additionally, it will provide identification and further characterization of potential sensitive populations to consider in future epidemiological and inhalation risk assessment studies.

S 1751b Interaction of Alpha-Synuclein with Divalent Metal Manganese Alters Disease Progression in Transgenic Models of Parkinson's Disease

D. S. Harischandra. *Iowa State University, Ames, IA.*

Parkinson's disease (PD) is a multifaceted neurodegenerative condition caused by complex interplay of genetic and environmental factors. Manganese (Mn), is considered a key inhaled pollutant implicated in environmentally linked PD as evidenced by epidemiological studies done on humans exposed to Mn through inhalation in mining, welding metals, and dry battery manufacturing settings. However, the exact molecular mechanisms underlying Mn induced protein aggregation or the role of pre-existing conditions such as gene mutations linked to PD are not well understood. In our experiments, we are evaluating the interaction of Mn and a-synuclein (aSyn), a key metal binding protein implicated in PD. Specifically we are evaluating the ability of aSyn gene mutations to modulate susceptibility to promote aggregation and accumulation of the aSyn protein as a key pathological feature of PD. We have used transgenic mouse models such as aSyn-A53T and newly developed MitoPark mouse model of PD to evaluate these gene-environment interaction in PD-like neurological disorders. After exposure to Mn, behavioral and neurological deficits were evaluated using locomotor, neurochemical and histological analysis. Data suggest that baring pre-existing conditions to neurodegenerative disorders would lead to differential expression in disease progression as evident by MitoPark mice exacerbating susceptiblity to Mn induce behavior deficits while aSyn-A53T show hypoactivity. Interestingly, female MitoPark mice were affected more severely than male MitoPark mice. Collectively, our data demonstrate that pre-existing mutations would intensify the susceptibility to environmental neurotoxicants such as Mn, and promote development of PD-like neurodegenerative disorders. (NIH grants ES19267and ES10586)

S 1752 Human Airway Epithelium: Ethinyl Estradiol-Mediated Sex Differences in Smokers and Non-Smokers

M. Rubelli. *University of North Carolina, Chapel Hill, Center for Environmental Medicine, Asthma, and Lung Biology, Chapel Hill, NC.*

Sex has been identified as a risk factor for a variety of lung diseases, including early onset COPD, where 70% of the patients are women. It is known that females are disproportionately affected, but the contribution of estradiol, the primary circulating estrogen in women, is unknown. While inhaled pollutants like cigarette smoke contribute to an increased number of patients with COPD, it does not explain the increased incidence in women. To investigate this question, we examined both genomic and non-genomic factors by which sex and smoking may both independently and concurrently contribute to sex-biased lung disease susceptibility. Nasal lavage and nasal turbinate scrape biopsies were collected from smokers and non-smokers of both sexes for this study. Samples were isolated from women during high and low circulating estrogen phases of the menstrual cycle. After RNA isolation, sex-associated and estradiol-dependent effects on mRNA expression were evaluated using nanoString nCounter analysis (NanoString Technologies, Seattle, WA). Changes in innate immune response to infection (Influenza A) were also evaluated with concomitant exposure to philologically relevant levels of ethinyl estradiol by PCR and ELISA in immortalized human bronchial epithelial cells (Beas-2B) as well as in primary nasal epithelial cells cultured at air-liquid interface. Estradiol mediated changes in epithelial gene expression, as well as the innate immune response, in female smokers compared to male smokers illustrate that sex as well as cigarette smoking may influence disease susceptibility.

S 1753 *In Utero* Secondhand Smoke Exposures Increase the Lungs' Susceptibility to Developing Emphysema-Related Respones As Adults

A. Noel. *Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Baton Rouge, LA.*

Second-hand smoke (SHS) is a main indoor air pollutant. An increasing number of epidemiological and experimental studies have associated exposure to SHS during pregnancy with adverse outcomes in newborns. As we have previously shown, *in utero* SHS exposure alone alters both lung function and structure in male mice, while only lung structure is affected in female mice, at least through 15 weeks of age. However, whether *in utero* SHS exposures influence adult susceptibility to develop emphysema has not been resolved. Here, we hypothesized that *in utero* SHS exposures will predispose adult mice to the development of elastase-induced emphysema. Pregnant Balb/c mice inhaled filtered air or 10 mg/m3 of SHS from gestation days 6-19. At 11 weeks of age, female offspring were instilled intratracheally with 0.1 U/g of elastase or saline. At 15 weeks, lung function, gene expression, inflammatory responses, oxidative stress and airspace enlargement were assessed. The lungs of mice exposed *in utero* and as adults to SHS, along with elastase treatment, showed significantly increased maximum dynamic airway resistance, which was associated with significant increases in the percentage of neutrophils and level of oxidative stress in the broncho-alveolar lavage fluid. Mice exposed *in utero* to SHS and treated as adults with elastase, as well as the combined *in utero* + adult SHS/elastase group, exhibited significant increases in alveolar airspace enlargement (mean linear intercept values of 173 and 203 µm, respectively vs. 66 µm for controls) and significant decreases in the surface area of the lungs. Furthermoore, elastase treatment caused up-regulation of MMP12 and down-regulation of TIMP1 & 2, Adam33 and Sirt1 gene expression. As emphysema pathogenesis involves protease-antiprotease imbalances and airspace enlargement, these experimental findings suggest that *in utero* SHS exposures increase the lungs' susceptibility to develop emphysema-related responses as adults.

S 1754 The Effect of Diesel Exhaust Exposure on Patients with Allergic Rhinitis–Implications for NK Cell Physiology

E. Pawlak. *University of North Carolina, Chapel Hill, Center for Environmental Medicine, Asthma, and Lung Biology, Chapel Hill, NC.*

Exposure to diesel exhaust has been shown to exacerbate respiratory inflammation in subjects with allergic rhinitis. Recently, natural killer (NK) cells have been implicated in the resolution of allergic inflammation via interaction with eosinophils. Previous work by our group has shown suppressed NK cell cytotoxic function and cytotoxic granule production in response to diesel exhaust exposure. We therefore hypothesize that a mechanism of susceptibility to increased respiratory inflammation following exposure to inhaled pollutants, in humans with allergic rhinitis, is the suppression of NK cell activity. 22 human volunteers with allergic rhinitis were exposed to diesel exhaust or clean air for 2 hours and challenged with the live attenuated influenza vaccine. Subjects exposed to diesel exhaust had significantly increased markers of eosinophil activation and decreased markers of NK cell chemotaxis over a 7–10 day time course post-challenge. Additionally, peripheral blood NK cells challenged *in vitro* with diesel exhaust particles had decreased cytotoxic activity against an eosinophil cell line. Taken together, these data suggest that the NK cell is an important regulator of allergic inflammation and this role is compromised with exposure to diesel exhaust. Exposure to traffic-related pollution, asthma, and viral infections frequently overlap, particularly in urban areas. Defining mechanisms for how these factors interact is critical to addressing the effects of environmental pollutants on human respiratory health.

1755 Inhaled Ozone Causes More Severe Lung Lesions in Hyperglycemic, Insulin-Resistant KKAy Mice As Compared to Normoglycemic, Insulin-Sensitive C57BL/6 Mice

D. N. Jackson-Humbles. *Michigan State University, Ann Arbor, MI.*
Sponsor: D.B. Miller.

Epidemiological studies suggest that diabetics are susceptible to the adverse health effects of air pollution. In this study we tested the hypothesis that repeated inhalation exposures to a common gaseous air pollutant, ozone (O3), will induce more severe lung lesions in mice with pre-existing hyperglycemia and insulin-resistance as compared to normoglycemic, insulin-sensitive mice. Male, normoglycemic C57BL/6 (C57) and hyperglycemic KKAy mice were exposed to 0 ppm (filtered air; FA) or 0.5 ppm O3, 4h/day, for 13 consecutive weekdays. After final exposure, mice underwent insulin tolerance tests (ITT) and were sacrificed 24 hours postexposure. Bronchoalveolar lavage fluid (BALF) was analyzed for inflammatory cells and cytokines. Lung tissues were processed for histological, morphometric, and RT-PCR analyses. FA or O3-exposed C57 mice had normal ITT values, insulin sensitivity, and normal fasting blood insulin levels unaltered by O3 exposure. Conversely, FA and O3-exposed KKAy mice had abnormal ITT values, insulin resistance (IR). Although all KKAy mice were hyperinsulinemic, fasting blood insulin levels were significantly reduced after O3 exposure. In KKAy mice, O3 induced greater inflammatory cells and elevated IL-5 in BALF along with severe centriacinar lung lesions that included marked eosinophilic and neutrophilic inflammation, epithelial hyperplasia, and peribronchiolar and alveolar fibrosis compared to similar but modest BALF and mild lung changes in C57 mice. Fibrotic lesions were associated with distinct eosinophilia and relative fold increases in Ccl11, Il13, and Mmp12 expression, compared to FA-exposed KKAy mice. Hyperglycemic KKAy mice, a type II diabetic model, had significantly greater O3-induced lung lesions as compared to similarly exposed, normoglycemic C57 mice. These results suggest type II diabetes or pre-diabetic conditions may increase susceptibility to adverse respiratory effects caused by elevated concentrations of ambient O3. Funded by US EPA RD83479701.

1756 Obesity As Risk Factor for Ozone-Induced Cardiopulmonary Effects and Metabolic Impairment

S. J. Snow. *Cardiopulmonary and Immunology Branch, US EPA, Durham, NC.*

As metabolic syndrome has become a global epidemic, a growing body of evidence indicates an association between air pollution and adverse health effects in susceptible populations with metabolic disorders. We hypothesized that the preexistence of type 2 diabetes and/or diet-induced obesity (DIO) would exacerbate O3-induced cardiopulmonary responses and induce metabolic impairment. Male Wistar and Goto-Kakizaki (GK) rats, a lean type 2 diabetic inbred model derived from Wistars, were fed a normal or high-cholesterol atherogenic Western diet for 12 weeks starting at 4 weeks of age. After the 12 week diet, rats were exposed to filtered air or O3. A number of metabolic and cardiopulmonary assessments were made to understand the dynamic changes. The atherogenic diet induced DIO in both strains as evidenced by increased body fat percentages and body weight. Multiple biomarkers of lung injury in bronchoalveolar lavage fluid were increased temporally following O3 exposure in both strains. O3-induced lung injury and inflammation were exacerbated in the GKs, indicating that an acute O3 exposure may augment pulmonary injury in diabetics. Although strain-related differences were apparent, these markers were not altered by DIO. Glucose tolerance testing was conducted and serum was collected following exposures to assess metabolic impairment. Preexistence of diabetes and obesity augmented O3-induced hyperglycemia and glucose intolerance. Metabolic serum markers were elevated following exposure, suggesting ozone-induced dyslipidemia and lipolysis. Collectively, these data demonstrate that exposure to ozone 1) leads to increased pulmonary damage in diabetic rats, 2) has a profound effect on glucose and lipid metabolism, and 3) augments metabolic impairment in animals with preexisting type 2 diabetes and DIO. These metabolic alterations might arise from neuronal stress mechanisms, and upon longer air pollution exposure, may lead to insulin resistance and accelerated diabetes.

1757a Systems Understanding of the Impact of the Nrf2 Pathway on Chemical Toxicity and Cell Fate

C. Corton[1] and T. Kensler[2]. [1]*US EPA, Durham, NC and* [2]*University of Pittsburgh, Pittsburgh, PA.*

The transcription factor Nrf2 plays a significant role in protecting cells from endogenous and exogenous stresses. Initial studies of the Nrf2 pathway focused on altered metabolism of toxins, leading to their enhanced detoxication and elimination. Continuing studies expanded this view of Nrf2 action to include pathways affecting recognition, repair, and removal of damaged macromolecules. Most recent studies have focused on the links between cellular metabolism and cell fate. Some of these actions are mediated through cross-talk with other signaling networks. This symposium will highlight recent exciting findings in the field that greatly extend our systems understanding of the function and regulation of Nrf2 using a broad range of omic tools. The first speaker will describe the first screen for genetic effectors of Nrf2 activity. The second speaker will discuss a novel screening strategy in a large gene expression compendium to identify signaling pathways that affect Nrf2. The third speaker will describe a unique role for Nrf2 in circadian rhythm. The fourth and fifth speakers will describe exciting work that links Nrf2, metabolic reprogramming, and cell fate. The session will be of interest to scientists interested in stress pathways and how omics tools can be applied to study mechanisms of toxicity.

1757b Imaging-Based RNA Interference Screening Identifies Novel Regulators of Nrf2 Signaling: Consequences for Drug-Induced Liver Injury

B. van de Water. *Leiden University, Leiden, Netherlands.*

The overall signaling components that control Nrf2 signaling remain largely elusive. We performed a high content imaging-based RNA interference screen using an Nrf2-dependent fluorescent reporter system: BAC-based Srxn1-GFP reporter cells. We screened for individual kinases, ubiquitinases and transcription factors that control Nrf2 activity. Candidate genes were further validated with different individual siRNA sequences as well as in BAC NQO1-GFP reporter cells by similar approaches. Hits were related to the control of Nrf2 stability using live cell imaging. Validated regulators were further evaluated for the modulation of Nrf2 activation caused by different compounds that cause drug-induced liver injury. Moreover, these regulators were tested for their modulation for the susceptibility towards cytotoxicity. The current work has identified a diverse panel of previously unknown determinants of Nrf2 pathway regulation and contributes to our overall understanding of the signaling networks that control Nrf2 activity in health and disease as well as drug safety.

1758 A Biomarker-Based Screen of a Large Gene Expression Compendium Reveals Regulation of Nrf2 by CAR and STAT5b

C. Corton. *US EPA, Durham, NC.*

Computational approaches were developed to identify factors that regulate Nrf2 in compendium of microarray profiles including >2000 comparisons which queried the effects of chemicals, genes, diets, and infectious agents on gene expression in the mouse liver. A gene expression biomarker of 48 genes was shown to accurately predict Nrf2 activation and was used to identify factors which resulted in a gene expression profile with significant correlation to the biomarker. A number of novel insights were made. Chemicals that activated the xenosensor constitutive activated receptor (CAR) consistently activated Nrf2 across hundreds of profiles, possibly downstream of Cyp-induced increases in oxidative stress. Nrf2 activation was also found to be negatively regulated by the growth hormone (GH)- and androgen-regulated transcription factor STAT5b, a transcription factor suppressed by CAR. Nrf2 was activated when STAT5b was suppressed in female mice vs. male mice, after exposure to estrogens, or in genetic mutants in which GH signaling was disrupted. A subset of the mutants that show STAT5b suppression and Nrf2 activation exhibit increased resistance to environmental stressors and increased longevity. This study describes a novel approach for understanding the network of factors that regulate the Nrf2 pathway and highlights novel interactions between Nrf2, CAR and STAT5b transcription factors. (This abstract does not represent EPA policy.)

1759 Closing the Loop: Defining the Role of Nrf2 in Clock Repression

T. Sutter. *University of Memphis, Memphis, TN.*

Nuclear erythroid factor 2 like 2 (Nrf2) is a transcription factor that regulates cellular adaptive responses to oxidation and electrophilic stresses. Clock and Bmal are core clock transcription factors that regulate circadian rhythms of physiology and behavior. As a heterodimer, Clock and Bmal bind E-box elements and regulate target genes including Period and Cryptochrome, major components of a negative feedback loop that drives daily oscillations of output genes. Recent work by others has shown that Nrf2 is an E-box-regulated target gene of clock. Through analysis of high-content data of liver response to Nrf2 activators, we identified chemical and Nrf2-genetic effects on multiple core clock transcripts. Using genetic and biochemical methods, we show that Nrf2 contributes to the feedback mechanism of the transcriptional repression of Bmal/Clock. These results place Nrf2 in a network regulating tissue and cellular integration of circadian rhythm, stress responses, and physiology such as metabolism.

1760 The Nrf2-Notch Axis: Expanding the Cell Fate Network

T. Kensler. *University of Pittsburgh, Pittsburgh, PA.*

Data emanating from gene expression microarray analyses comparing Nrf2-disrupted and wild-type mouse embryonic fibroblasts (MEF) showed that expression of Notch1 and Notch-signaling related genes were decreased in Nrf2-disrupted cells. A functional ARE has been identified upstream of the Notch1 major transcription start site. Furthermore, an Rbpjk binding site is conserved on the promoters of Nrf2 among animal species. Notch1 is the one of the transmembrane Notch family receptors, which drives Notch-signaling together with the Rbpjk transcription factor. After canonically accepting ligands such as Jags and Deltas, the receptor undergoes cleavage to yield the Notch intracellular domain which translocates to the nucleus. Recent studies using conditional knockout mice indicate that Notch1 as well as Notch2 play important roles post-natally in liver development and in maintenance of hepatic function. This talk will summarize current understanding of the role of reciprocal transcriptional regulation between Nrf2 and Notch in adult liver from studies using Nrf2, Keap1, and Notch1 genetically engineered mice.

1761 Crosstalk Between Regulation of Redox Balance and Cell Proliferation by NRF2

H. Motohashi. *Tohoku University, Miyagi, Japan*. Sponsor: C. Corton.

NRF2 is a master transcriptional activator playing a critical role in the defense mechanism against oxidative insults. NRF2 activates genes encoding cytoprotective enzymes and antioxidant proteins in response to electrophiles and reactive oxygen species (ROS) of exogenous and endogenous origins. Under unstressed conditions, NRF2 is constantly ubiquitinated by KEAP1 and degraded in the proteasome. During exposure to electrophiles or ROS, KEAP1 is inactivated, and NRF2 is stabilized. Consequently, NRF2 activates transcription, conferring resistance against xenobiotic and oxidative stress. While NRF2 activation is beneficial to our health, NRF2 is responsible for the malignant progression of cancers. We found that NRF2 not only enhances survival of cancers by activating cytoprotective genes but also redirects glucose and glutamine into anabolic pathways by activating metabolic genes, which are advantageous for cancer proliferation. Enhanced activity of PI3K-AKT signaling enables NRF2 to induce the metabolic genes and modulate metabolism. We observed that NRF2 stabilization under the sustained activation of PI3K-AKT pathway in hepatocytes results in liver enlargement. Intriguingly, it was accompanied by the marked increase of bile duct-like structures, suggesting that the robust activation of NRF2 affects the cell type specification during liver development besides cell proliferation. We also found that NRF2 activation promotes hepatocyte proliferation during compensatory liver hypertrophy after partial portal vein ligation, implying that NRF2 contributes to proliferation under the transient and physiological enhancement of proliferative signals. These results suggest that the functional potentiation of NRF2 by proliferative signals mediates metabolic reprogramming and boosts cell proliferation.

1762a Unknown, Unknowns: Exploring the Unidentified Fraction of Complex Mixtures

C. Rider[1] and B. Hannas[2]. [1]*NTP/NIEHS, Research Triangle Park, NC and* [2]*The Dow Chemical Company, Midland, MI.*

Assessing risk from exposure to complex mixtures, ranging from botanical dietary supplements to traffic pollution, is exceptionally challenging. A hallmark of complex chemical mixtures is the presence of unidentified mixture mass. The contribution of these unknown chemicals is difficult to characterize. In terms of exposure, there is a prevailing tendency to measure only a select subset of target constituents (i.e., those with reason for toxicological concern or those that are present at the highest concentrations) within a complex mixture, resulting in a significant unidentified fraction. Paradoxically, toxicological assessments are typically limited to chemicals for which exposure data are available. These targeted strategies were developed to focus attention on the chemicals of greatest concern, but they implicitly overlook the unidentified fraction, which can represent the largest mass of the mixture. In risk assessment of chemical mixtures, component-based approaches, which deal exclusively with mixture constituents for which exposure and toxicological data are available, are far more common than those that consider whole mixtures. This is based on both a lack of available whole mixtures data and uncertainties in extrapolating from a reference mixture to a mixture of concern. In contrast to component-based approaches, whole mixture approaches account for the unknown mixture mass but do not identify the impact attributable to the unknown fraction. In this workshop, the unidentified fraction of complex mixtures will be brought to the forefront. Advances in chemical analysis, toxicology, and whole mixtures risk assessment will be discussed in the context of exploring the unknown portion of complex mixtures. Speakers from academia, government, and industry with diverse expertise will discuss the latest techniques for exploring complex mixtures, including the unidentified fraction. Throughout the discussion, areas that require further development or refinement will be identified. Finally, comparison of the current standard (i.e., targeted approaches) with more global whole mixtures methods will be made in terms of data needs, feasibility, and added value.

1762b Bridging Chemical Exposures Technology with Bioactivity through Combining Passive Wristband Samplers and Bioassays to Better Understand Mixtures

K. Anderson. *Oregon State University, Corvallis, OR*. Sponsor: C. Rider.

Accurately assessing the breadth of a person's exposures to environmental toxicants is central to the challenge of studying environmental health effects. The lack of low cost, easy-to-use personal sampling technology hinders many situations where sampling is challenging. Personal sampling wristband technology (BRIDGES: Biological Response Indicator Devices for Gauging Environmental Stressors) was developed specifically because Public Health researchers and Community partners were enthusiastic about its broad potential to fill a key gap in Public Health. Our passive wristband sampler was developed to provide a relevant, quantifiable assessment of bioavailable chemical exposure. Volatile and semi-volatile organic chemicals (boiling point <450?C) can be quantified from the wristband. We screen the wristband, in a single method, for over 1,400 organic compounds, and we can quantify several 100 analytes such as PAHs, pesticides, flame retardants, PCBs, and many consumer products. To separate the biology and the environment in an a priori fashion is to reduce the system to a level below the phenomena of interest. We demonstrate that the wristband extract can be used directly in bioassays to assess the bioactivity of actual chemical mixtures from an individual's exposures. We are able to further chemically divide, fractionate, the wristband extract and assess the bioactivity of each fraction. The effects directed process of assessing the mixture provides insight into the mixture and the additive or non-additive nature and the chemicals associated with each fraction and the original mixture. Presented here are some examples of pilot studies. In western Africa in a farming community study we found that wristbands contained 2 to 10 pesticides, with deltamethrin and cypermethrin most frequently detected. Journeyman roofers wore the wristbands for 8 hrs and we quantified 31 polycyclic aromatic hydrocarbons. Overall, we have found that people are actually eager to wear the wristband and we have 85-100% compliance in studies to date.

1763 Predicting Network Activity from High-Throughput Metabolomics

D. Jones. *Emory University, Atlanta, GA.*

In complement to untargeted chemistry approaches to explore complex mixtures, metabolomics can offer an agnostic assessment of the biological response of an organism to a complex mixture. A striking potential advantage of metabolomics in toxicological assessments of complex mixtures is that both hypothesized and unanticipated changes in the physiology of the organism are detectable. Mixtures experiments are increasingly focusing on the low dose/low effect region where metabolome changes that occur in advance of overt adverse effects may provide information on resiliency and sensitivity. Untargeted metabolomics approaches use liquid chromatography/mass spectrometry (LC/MS) to measure thousands of metabolites. However, a major challenge has been in identification of the observed metabolites, which can be a challenging process. Recent advances in computational biology have increased the predictive power of this technology. Machine learning techniques have been used to separate signal from noise and increase identification potential.

1764 Combining Toxicological and Chemical Characterization of Complex Mixtures to Understand the Impact of the Unknown Fraction

J. E. Simmons. *US EPA, Research Triangle Park, NC.*

Toxicological assessment of adverse health outcomes associated with exposure to complex mixtures provides an integrated response of the organism (or *in vitro* test system) that accounts for additivity among the components (both dose and response) as well as any greater than or less than additive interactions. However, toxicological information alone is often inadequate for extrapolation to other mixtures within the same class, such as mixtures formed during oxidant disinfection of water, or those formed during fuel combustion. Examples from combined chemical and toxicological characterization of a variety of complex chemical mixtures, including hazardous wastes, disinfection byproducts (DBPs), chemicals of emerging concern in surface and treated water, and air pollution mixtures will be used to illustrate how failure to account for the unidentified fraction potentially results in inaccurate toxic potency estimates. Risk remediation efforts benefit from an understanding of the mixture components that contribute most to the toxicity of the mixture. Two recently developed methods that require both concentration and toxicity data, the expected component contribution score (ECC) and a method based on regression modeling techniques, are illustrated with complex (DBP) and defined (DBP, carbamate) mixtures. The ECC requires knowledge of the concentrations and the relative toxic potencies of the component chemicals. The regression modeling approach requires dose-response, concentration and toxicity data. Additionally, a data intensive assessment strategy for determining the contributions of individual chemicals, additive and nonadditive interactions and the unknown fraction to mixture toxicity will be illustrated. (This abstract does not reflect EPA policy.)

1765 The Use of Statistical Models to Predict Systemic and Developmental Toxicity of Crude Oil As an Example of Complex Petroleum Substances

R. H. McKee. *American Petroleum Institute, Annandale, NJ.*

Petroleum (commonly called crude oil) is a complex substance primarily composed of hydrocarbon constituents. Based on the results of previous toxicological studies as well as occupational experience, the principal acute toxicological hazards are those associated with exposure by inhalation to volatile hydrocarbon constituents and hydrogen sulfide, and chronic hazards are associated with inhalation exposure to benzene and dermal exposure to polycyclic aromatic compounds. The current assessment was an attempt to characterize the potential for repeated dose and/or developmental effects of crude oils following dermal exposures and to generalize the conclusions across a broad range of crude oils from different sources. Statistical models were used to predict the potential for repeated dose and developmental toxicity from compositional information. The model predictions indicated that the empirical data from previously tested crude oils approximated a "worst case" situation, and that the data from previously tested crude oils could be used as a reasonable basis for characterizing the repeated dose and developmental toxicological hazards of crude oils in general.

1766 Whole Mixtures Risk Assessment: Considering Sufficient Similarity

G. Rice. *US EPA, Cincinnati, OH.* Sponsor: C. Rider.

Many complex mixtures contain hundreds of component chemicals, including an unidentified fraction. The mixture composition can vary over time, place or different conditions under which the mixture is produced or released into the environment. When assessing human health risks associated with complex environmental mixtures, risk assessors prefer whole mixtures data and risk assessment approaches to component-based approaches that rely on single chemical data, because they require fewer assumptions and account for any interactions among chemical constituents, including mixture constituents within the unidentified fraction. Due to limitations in available whole mixture data, it is often necessary to utilize data from a reference mixture that is determined to be sufficiently similar. However, methods for determining sufficient similarity of a reference whole mixture to the mixture(s) of interest have not been widely applied to date. Examples of whole mixtures approaches that rely on multifactorial comparisons (e.g., principal component analysis, bootstrap hypothesis test procedures) of chemical and biological data from multiple mixtures will be presented for different complex mixtures including drinking water disinfectant by-products. Using such methods, mixtures from various water treatment plants were compared. The water source (ground versus surface) was found to have a greater impact on mixture variability than other factors such as whether samples were obtained from finished or distribution system water. These methods offer promise for increased application of whole mixtures risk assessment procedures. (This abstract does not reflect EPA policy.)

1767a Bioactivity-Based Margin of Exposure Safety Assessment: The Next Stop along the Road to 21st Century Safety Assessments

J. C. Rowlands[1] and N. Walker[2]. [1]*The Dow Chemical Company, Midland, MI and* [2]*National Toxicology Program at NIEHS, Research Triangle Park, NC.*

The ultimate goal of toxicity testing in the 21st century is the reduction and eventual replacement of animal-based toxicity testing with *in vitro* assays primarily using cultured human cells coupled with human exposure information. While significant progress has been made toward this end, there are numerous challenges that must be overcome before the final goal can be realized. Nevertheless, there are important regulatory decisions that these alternative approaches can be applied to that can serve as interim approaches to safety assessment. By using margin of exposure (MOE) as the primary metric for safety assessment, bioactivities measured in alternative approaches combined with human exposure determined through *in vitro* to *in vivo* extrapolations (IVIVE) or human biomonitoring studies can be used for regulatory decisions such as testing prioritization, emergency response assessments, and safety assessments. Bioactivity-based MOE provides a risk-based and animal-sparing approach to evaluate chemical safety, drawing broadly from previous experience but incorporating alternative 21st century alternative approaches to increase efficiency and reduce animal usage. The workshop will comprise a series of highly focused presentations that evaluate using bioassay-based points of departure for regulatory decision-making in chemical risk assessment. Each speaker will demonstrate how their recommended approach provides a practical solution to specific regulatory decision.

1767b Biological Canaries in the Coal Mine: Strategic Implications for Utilizing Bioactivity-Based Margins of Exposures in Human Health Risk Assessments

N. Walker. *National Toxicology Program at NIEHS, Research Triangle Park, NC.*

Traditionally, risk assessments has been an integration of hazard identification, dose response and exposure assessment. However, the number of chemicals, their combinations in environmental mixtures, and the low throughput nature of traditional disease specific toxicology models, limits the timely assessment of exposures of potential concern. The use of "biological activity" in sentinel organs/tissues from shorter term studies has been used to identify exposures or doses that occur in the same region as those benchmark doses identified from dose response assessment of apical hazards identified in toxicology studies following longer term exposures. As such while the relationship of a biological response to an actual hazard may be uncertain, this approach may be employed to identify levels of exposure that are likely be sufficiently protective of the hazards. The advantage of such an "exposure based" hazard assess-

ment strategy is that by focusing on the lower end of the dose response curve with potentially more sensitive molecular endpoints, some of the uncertainties associated with high dose-low dose extrapolation and use of "hazard–specific" toxicological models are obviated. The pitfalls associated with this approach are that a new set of uncertainties is now employed, including the assumption that the chosen sentinel organ response is the most sensitive one. Ideally the use of such an approach would be coupled with other data streams in a broader strategy. These strategies would be applied to reduce some of the uncertainties associated with prioritization schemes based on higher throughput *in vitro* approaches, together with an assessment of how *in vitro* doses related to likely real world exposure. In the near–term, candidates for assessment using such an approach would be for chemicals where the predicted margin of exposure would neither be too small therefore and ought to go directly to a more comprehensive hazard assessment, or too large, where the priority to commit resources for in depth study would be low.

W 1768 An Exposure: Activity Profiling Method for Interpreting High-Throughput Screening Data for Estrogenic Activity

R. Becker. *American Chemistry Council, Washington, DC.*

Rapid high throughput *in vitro* screening (HTS) assays can be used to screen substances to characterize activities and dose-responses for estrogen and androgen endpoints. But such activity measurements alone fall short for the purposes of priority setting or screening unless the relevant exposure context is considered. Our initial (Becker RA et al. 2014, 2015) margin of exposure (MOE) approach for endocrine used the exposure:activity ratio (EAR= 1/MOE) based on human exposure estimates and activity metrics of AC50 (from *in vitro* assays) and BMD (from *in vivo* assays)). EPA developed a similar MOE approach, the Integrated Bioactivity Exposure Ranking (IBER) method, using a scoring system based on summing AUC values for activities from ToxCast and Tox21 assays measuring specific elements of the estrogen pathway and high throughput human exposure modeling. Relative endocrine exposure:activity quotients (REEAQ) provide additional context for understanding the significance of these MOE metrics; REEAQs for estrogen activity are derived by comparing chemical-specific EARs and IBERs to such values for the ubiquitous dietary phytoestrogen, genistein (GEN). Although the activity of a substance in HTS-endocrine assays is not a measure of health hazard or risk, understanding how such an activity for a specific compound compares to human exposures and the exposure:activity profile of benchmark substances, such as GEN, provides much needed context for priority setting and other decisions. This presentation will compare and contrast the EAR and IBER MOE approaches, discuss threshold motifs and raise the question whether consideration should also be given to developing activity cut off values for each hormonal system, such that substances below such cutoffs would be directly deprioritized.

W 1769 Moving Forward with Canada's Chemicals Management Plan: Opportunities for the Integration of High-Throughput Technologies in a Margin of Exposure Approach to Support Risk Assessment Activities

T. Barton-Maclaren. *Hazard Methodology Division, Health Canada, Ottawa, Canada.* Sponsor: J.C. Rowlands.

Canada's Chemicals Management Plan (CMP) announced in 2006 set clear priorities for assessing and managing substances used in Canada. Health Canada continues to make progress on the commitment to address 4300 chemicals on the Domestic Substances List by 2020 in a manner that is protective of human health and the environment. A key challenge includes limited empirical data sets for a subset of remaining priorities. Traditional whole animal testing is not feasible for all of the chemicals requiring evaluation under the CMP. Further, there is a need to be able to accommodate substances and substance groupings with variable toxicological information, including data from emerging methodologies. The utility of high-throughput screening (HTS) data is currently being explored for a range of applications under the CMP including dose-response analysis, hazard identification, and to inform screening level risk assessments based on a margin of exposure (MOE) approach. A screening approach to facilitate the integration of HTS data into the risk assessment paradigm has been proposed by the US EPA where *in vitro* bioactivity is used as a point of departure to derive a bioactivity-exposure ratio (BER); a metric analogous to the MOE. Canada's CMP continues to harvest new innovations in regulatory science – moving to higher output, lower cost methods to predict the potential for hazard and risk of data-poor chemicals. This presentation will provide an overview with examples of Health Canada's early investigations aiming to establish proof-of –concept and determine the level of confidence in the approach for integrating high-throughput technologies to derive *in vitro* based MOEs based on bioactivity and estimates of human exposure for future human health risk assessment activities under the CMP. New approaches come with challenges. The pragmatic and technical considerations facing the transition to the integration of next generation toxicity testing tools to support work under Canada's CMP will be discussed.

W 1770 Predicting the Benchmark Dose for Dioxin-Induced Liver Tumors from the Gene Expression Changes at 28-Days

J. C. Rowlands. *The Dow Chemical Company, Midland, MI.*

Liver tumor promotion in rodents is a well characterized affect from exposure to dioxin. The key events for the mode of action for liver tumor induction have been identified and been utilized to support an AOP for liver tumor promotion. Dose-related gene expression analysis from a 28-day dioxin exposure study conducted in rats was used to calculate the standard benchmark dose (BMD) for cellular biological processes for key events. A comparison of the transcriptional BMD values with those for the traditional cancer apical endpoint showed a high degree of correlation for specific cellular biological processes. The results indicate that there is a high degree of fidelity between the predicted BMD for the tumor response at 2 years and the calculated transcriptomic-based BMD from a short term study. By comparing the human exposure data, to the transcriptional BMD, a margin of exposure can be calculated. The correlation between the BMD values for the transcriptional and apical endpoints and the ability to calculate a margin of exposure analysis suggest that transcriptional BMD values may be a useful approach as potential points of departure for risk assessment of chemicals.

W 1771 Application of Genomic Benchmark Dose Analysis in the NTP Response to the Elk River Chemical Spill

S. Auerbach. *National Toxicology Program at NIEHS, Research Triangle Park, NC.*

In January 2014, approximately 10,000 gallons of a liquid used to wash coal and remove impurities was spilled from a leaking tank into the Elk River in West Virginia. The primary constituent of this bill was 4-methylcyclohexanemethanol (MCHM, CASRN 34885-03-5). An initial review of the toxicology dossier on the chemicals in the spill indicated many had limited toxicological characterization. In order to provide a rapid estimate of the *in vivo* biological point of departure NTP performed a five day repeat dose toxicity study with a broad dose range in male rats followed by gene expression analysis of liver and kidney. Individual genes were fit to a variety of dose response models using BMDexpress. Genes were then grouped into pathways and pathway level points of departure were determined. A review of the benchmark dose values indicated the findings were quantitatively consistent with previously performed guideline studies. The findings suggest that short-term *in vivo* genomic dose response analysis may be used to rapidly identify screening level points of departure for rapid response risk assessment.

W 1772a Maternal Exposure to Nanoparticles—How Does It Affect the Fetus? Status, Mechanisms, and Future Directions

F. R. Cassee[1] and S. L. Makris[2]. [1]*National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands and* [2]*US EPA, Washington, DC.*

Information on the effects of maternal exposure and possible effects on the fetus to small particles (nanometer range) is scarce and fragmented. Yet both epidemiological and toxicological studies suggest that maternal exposure to NP can cause adverse health effects on embryonic development such as increased number of terminated pregnancies (Hougaard et al., 2015). In this workshop, we aim to present the latest scientific information on reproductive and developmental effects of nano-sized particles, either from environmental relevant exposure (i.e., diesel engine exhaust) or engineered nanoparticles (single-walled carbon nanotubes, silver, cadmium oxide). This workshop will include a panel discussion at the end on relevance of dose determination, animal model appropriateness for humans, and what the next steps are to understanding human risk.

1772b Introduction: Reasons for Concern?

F. R. Cassee. *National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands.*

This introduction to nanoparticles covers a definition and description, potential exposures to humans via the environment and occupational settings and data from biodistribution studies that justify assessing adverse responses in embryos and fetuses. Inhalation exposure can result in maternal lung (and systemic) oxidative stress, and inflammation. Inflammation has been mentioned as the greatest enemy of pregnancy. The effects highly depend on when exposure occurs during development. For some widely used industrial nanomaterials, no new safety testing may be required for the nano-form because data are already available for the conventional form with the same chemical composition. This may not be a desirable situation either, because physicochemical properties of nanomaterials are known to affect the outcome of many kinetic biodistribution and toxicity studies. Maternal exposure to airborne pollution in humans, mainly through inhalation, has been shown to induce intra uterine growth retardation associated with decreased placental weight. Evidence of an association between lung exposure to nanoparticles, systemic inflammation and fetal abnormalities is however speculative, as only a few circumstantial, reports on nanomaterials have addressed this topic. Slightly more evidence is available from air pollution studies in animals or as part of observational studies. For example, exposure of rats to urban air pollution with a high content of particles (PM2.5, 600 microg/m3) before and during pregnancy increased the cytokine of interleukin 4 (IL-4) in the fetal part of the placenta, indicating a placental inflammatory reaction. The largest body of evidence relates to the associations between maternal exposure to ambient particles and effects on birth weight (corrected for gestational age). In a meta-analysis of studies in 14 centers, an increase by 10 microg/m3 in PM10 concentration was associated with a decrease of 9 g in mean birth weight, and an odds-ratio of 1.02 in risk for term low birth weight. The likelihood of direct or indirect effect on the fetus as a result of inhalation exposure to nanoparticles is an important consideration.

1773 The Reprotoxic Effect of Engineered Nanoparticles, *In Vivo* and *In Vitro*

L. Campagnolo. *University of Rome Tor Vergata, Rome, Italy.* Sponsor: F. Cassee.

In recent years interest for possible adverse effects after exposure to consumer relevant engineered nanoparticles (NP) during pregnancy has dramatically increased, probably in consideration of the higher susceptibility of developing tissues to any toxicant and the associated social implications. We have recently demonstrated for the first time that mouse exposure to low doses (down to 100 ng/mouse) of Single Walled Carbon Nano Tubes (SWCNTs) early in pregnancy (Gestational Day5.5, when no placenta is yet formed) induced severe embryo alterations, impaired placental vascularization and increased oxidative stress. More recently, we identified low amounts of fluorescently labelled SWCNT in placenta and in foetal membranes indicating the ability of NP to reach and distribute to these organs, but did not detect NP in the embryo, suggesting that the adverse effect might be secondary to placental alteration. Considering that in humans, as well as in rats and mice, placental permeability increases over gestation, we have further extended our analysis by investigating biodistribution and foetal effects of consumer relevant AgNP during the first 15 days of gestation, a critical window for embryo development in rodents. Pregnant mouse females were exposed by inhalation to freshly prepared 18nm AgNP (640 microg/m3 for 1 or 4 hrs per day). NP were identified and quantitated in the placenta and embryos by TEM, EDX and Single Particle ICP-MS analysis. In the placenta 0.0232% of the pulmonary concentration was measured, whereas AgNP in the embryo were barely detectable. However, 0.0116% of the pulmonary concentration was detected in resorptions. Interestingly, EDX microanalysis was complementary to SP-ICP-MS, giving undisputable evidence of AgNP in the exposed embryos. Of note, we did not observe significant differences in terms of maternal body weight gain, number of embryos/female, number of malformed embryos and expression of inflammatory cytokines between the groups with the exception of an increased number of resorption in the group that was exposed 4 h a day for 15 days. Although differences exist in placental and embryonic development between humans and rodents, the mouse has been successfully used to predict human embryotoxicity for drugs and chemicals, suggesting that this model might be exploited also to evaluate nanoparticle embryotoxicity.

1774 Maternal Airway Exposure to Nanoparticles in Pregnancy–Effects and Lessons Learned from Rodent Models

K. S. Hougaard. *National Center for the Working Environment, Copenhagen, Denmark.* Sponsor: F. Cassee.

Study of air pollution indicates that minute particles may adversely interfere with pregnancy and fetal development. Nanotechnology may potentially increase the overall particle burden in air and introduces particles with novel characteristics and surface reactivity, to workers and consumers. From the lungs nanoparticles may translocate to the placenta and fetus and here exert their effects directly. Alternatively, airway inflammation may translate into maternal low grade systemic inflammation, a potent modulator of pregnancy and fetal development. To evaluate safety for pregnant women, we studied developmental toxicity of engineered nanoparticles. Mice were exposed by inhalation, 1 hr per day, on GD 9-18, to 40 mg/m3 of titanium dioxide (17 nm, UV-titan L-181, Kemira) or carbon black (14 nm, Printex90, Degussa), or by intratracheal instillations to the corresponding deposited dose of Printex90 (268 µg total). Exposures substantially altered gene expression in livers from newborn F1, more so in females, e.g., relating to retionoic acid and metabolic function, and Printex90 furthermore increased DNA strand breaks as late as 50 days after birth. Exposures altered F1 behavior in the open field test, UV-titan also affected the female startle response. Exposures were associated with changes in sperm counts, for UV Titan in F1 males, for Printex90 in F2-males bred from exposed F1 males (but not exposed F1 females). To investigate also preconceptional exposure, we instilled naïve females once with 67 µg of multiwalled carbon nanotubes (Ø 10 nm, OECD NM-400). Exposure interfered with estrous cyclicity. Offspring from mating with an unexposed male did not show alterations in the open field or sperm count. When offspring were immunized with allergen, the specific antibody production was significantly decreased at 11 weeks of age. All exposures left pregnancy and lactational measures unaltered, despite pronounced lung inflammation in exposed dams. Thus, observed adverse effects mainly relate to functional changes in offspring physiology, even a long time after birth. This fosters concern since the effects were observed close to common 8 hr time weighted occupational exposure limits in the USA and Europe. Furthermore, functional outcomes (other than male reproductive function) are rarely addressed in developmental studies of industrial chemicals.

1775 Exposure to Nanoparticles During Pregnancy Can Impact Obstetric Outcomes and Early-Life Development in a Mouse Model: A Tale of Two Metals

J. T. Zelikoff. *New York University School of Medicine, Tuxedo, NY.*

The effects of nanoparticle (NP) exposure during pregnancy on the fetus and neonate are poorly studied and thus poorly understood. For these studies, timed-pregnant CD1 mice were exposed throughout gestation, via the pulmonary exposure route, to one of two medically- and industrially-important metal NPs, including cadmium oxide (CdO) and gold citrate and effects on the dam, obstetric parameters and fetal/neonatal growth were examined. Results demonstrated that exposure to relatively inert gold citrate NPs every second day from gestational day (GD) 4.4-16.5 and then either euthanized on GD17.5 or allowed to give birth had no adverse effects on maternal reproductive health or obstetric outcomes. However, 3-wk-old males had a significantly larger liver than their sex-matched control counterpart which was similar to that reported for directly-exposed adult mice. In contrast to the limited effects observed following prenatal exposure to AuNP, maternal exposure to similarly-sized CdO NP during pregnancy decreased fecundity, increased placental weight, decreased neonatal growth and increased urinary kidney injury marker (KIM)-1 expression during gestation and early during post-natal development. As commercial uses for nanotechnology continue to expand throughout the world, risks for unintentional exposure in the workplace and intentional exposure associated with medical strategies, increase. This presentation demonstrates the public health implications, particularly to already vulnerable sub-populations of pregnant women and their newborne of two commonly-used metal nanoparticles.

1776 Distribution and Accumulation of 10 nm Silver Nanoparticles in Maternal Tissues and Visceral Yolk Sac of Pregnant Mice, and a Potential Effect on Embryo Growth

P. L. Goering. *US FDA, CDRH, Silver Spring, MD.*

Silver nanoparticles (AgNPs) are currently the most extensively used nanomaterial in consumer products, and are utilized in nanomedicine, e.g., medical devices. To address knowledge gaps on effects of prenatal exposure to AgNPs, Ag distribution in pregnant mice and embryos/fetuses was examined following i.v. injection of 10nm AgNPs at a dose of 66 microg Ag/mouse (= 2.2 mg Ag/kg bw or 0.73 mg Ag/kg bw/day) to pregnant mice on gestation days (GDs) 7, 8, and 9. Selected maternal tissues and all embryos/fetuses were harvested on GD10 or GD16 for analysis of [Ag], intracellular AgNP localization, and gross examination of embryo/fetal morphology. AgNP administration resulted in Ag detected in all tissues examined, with highest concentrations in maternal liver and spleen and visceral yolk sac (VYS), and lowest concentrations measured in embryos/fetuses. Ag concentrations in tissues ranged from 0.03 ng Ag/mg tissue (embryos) to 2.57 ng Ag/mg tissue (maternal liver) at GD10, and 0.02 ng Ag/mg tissue (embryos) to 0.896 ng Ag/mg tissue (maternal liver) at GD16. Hyperspectral imaging confirmed the presence of AgNP aggregates in VYS and maternal liver, kidney, and spleen. No treatment-related tissue abnormalities were seen in maternal tissues, extra-embryonic tissues, and embryo/fetus; however, 8/10 AgNP-treated pregnant mice had one or more GD10 embryos that appeared small for their age (i.e. Theiler stage 13 [GD8.5] or younger) compared to 1/11 control mice, suggesting a possible effect of AgNP administration on embryo growth. In conclusion, i.v injection of 10 nm AgNPs led to appreciable Ag accumulation in maternal liver and spleen, and extra-embryonic tissues (i.e. VYS), but not embryos. High [Ag] in VYS and low [Ag] in embryos suggests that VYS acts as an effective barrier to the transport of Ag to embryos. Ag exposure resulting from the use of AgNP-containing medical devices would likely be at low levels; however, data on actual AgNP levels released are limited. Such data will be useful for risk assessments to provide estimates of patient exposures that can be compared to a tolerable intake level derived from animal or clinical studies.

1777 Potential Developmental and Early-Life Health Effects of Nanomaterials: Data Gaps and Research Needs for Risk Assessment

M. R. Gwinn. *National Center for Environmental Assessment (NCEA), US EPA, Washington, DC.*

Although research examining the toxicology of nanomaterials has been ongoing for many years, early studies largely focus on respiratory effects, and are limited by lack of appropriate dose metrics and a limited understanding of the role of the physicochemical properties of nanomaterials on the observed effects. More recently, efforts have been made to better understand other toxicities following exposure to nanomaterials, but large data gaps still remain, particularly related to the fetal impacts of maternal exposures. These data limitations impact the ability to adequately assess the risks associated with nanomaterials, making it difficult to evaluate nanomaterials beyond a case-by-case basis. As the use of nanomaterials increases rapidly in consumer products, there is an even greater need to understand the complexities of the issues surrounding the potential exposures to nanomaterials and their effects during development and early life. Current issues related to the risk assessment of nanomaterials include the lack of toxicokinetic data on transplacental transfer of nanomaterials to the fetus and the lack of understanding of the mechanisms of the potential direct and indirect fetal effects following maternal exposure to nanomaterials. For example, there is limited information on the amount of silver nanoparticles released from consumer products or medical devices that may directly or indirectly lead to fetal effects following maternal exposures. Further discussion is needed on the appropriate design of future toxicity studies to inform these issues. DISCLAIMER: The views expressed are those of the author and do not necessarily represent the views and/or policies of the Environmental Protection Agency.

1778a Multi-Omics in Predictive Toxicology: Development and Application in Environmental Monitoring Programs

S. Huang[1] and A. Mehinto[2]. [1]*AXYS Analytical Services Ltd., Sidney, BC, Canada and* [2]*Southern California Coastal Water Research Project, Costa Mesa, CA.*

The development of diverse omics technologies holds promise for understanding adverse outcomes induced by exposure to environmental chemicals. Omics technologies offer an efficient and high-throughput alternative to conventional toxicity testing, which is often not sensitive enough to detect effects of low-level chemical exposures and environmental mixtures. By evaluating changes in RNAs, proteins, and/or metabolite levels, omics technologies can inform on molecular initiating events as well as key pathways on the trajectory to adverse outcomes for exposed animals. This approach, known as adverse outcome pathway (AOP) analysis, has gained considerable interest recently through OECD and US EPA initiatives. Molecular biomarkers and fingerprints identified using this approach will be useful to identify the types of toxicants present in the environment and predict adverse health outcomes in humans and animals. The integration of the various omics technologies in environmental monitoring programs is powerful for toxicity assessments that can be used by government, industry, and academia to better define risk characterization. This workshop will focus on the development and application of omics techniques to investigate environmental issues. The speakers will present research studies that utilized omics technologies to identify chemical effects at different biological levels to develop AOPs. Their results will be used to discuss how omics data can be incorporated in monitoring programs and risk assessment.

1778b Adverse Outcome Pathways and Enhancing the Role of Genomics in Chemical Risk Assessment

G. Ankley. *US EPA, Duluth, MN.* Sponsor: S. Huang.

Historically, ecological risk assessments for chemicals have been largely based on toxicity data from whole animal tests with apical endpoints such as survival, growth and reproduction. This type of testing is costly and time-consuming. At the same time, regulatory programs throughout the world increasingly are requiring data for more chemicals and assessment scenarios. To meet this need, toxicologists are more commonly employing predictive toxicology tools which utilize mechanistic, pathway-based data that have not typically been used in the past for risk assessment applications. The adverse outcome pathway (AOP) concept provides a framework that explicitly depicts linkages between molecular/biochemical/histological and apical endpoints thereby enhancing the use of mechanistic data (including genomic information) for chemical risk assessments. This talk will provide an overview of the need for, and application of predictive toxicology tools to chemical risk assessment; in particular, the role of genomics in the development and implementation of AOPs will be addressed.

1779 Using Changes in the Transcriptome and Promoter Methylation to Explain Benzo[a] pyrene-Mediated Developmental Adverse Outcomes

K. Willett. *University of Mississippi, University, MS.*

Benzo[a]pyrene (BaP) is a well-known but mechanistically complicated carcinogen, reproductive and developmental toxicant. In zebrafish (*Danio rerio*), waterborne or dietary BaP/PAH exposure causes developmental adverse outcomes including growth reduction, neonatal death, cardiac dysfunction, skeletal abnormalities, craniofacial and fin deformities. In an effort to mechanistically explain these toxicities, we performed genome-wide transcriptional analysis and discovered differential gene expression and exon usage in zebrafish at two important developmental stages. Additionally, we measured both global and gene specific promoter methylation by multiplex deep sequencing of 21 genes known for their role in human diseases. Consistent with previous findings on the mechanisms of BaP toxicity, we found that BaP impaired canonical pathways including xenobiotic metabolism signaling, Nrf2-mediated oxidative stress response, glutathione-mediated detoxification, AHR signaling, and estrogen biosynthesis pathways. Novel differentially regulated and disease-related pathways included Notch, melatonin and ApoE pathways. Alternatively spliced myosin and collagen genes may be involved in BaP-induced growth deficits. In BaP-exposed 96 hr post fertilization larvae, global DNA methylation was significantly decreased and ten genes (6 hyper- and 4 hypomethylated) showed >10% change in CG methylation. Collectively, this presentation will bring together promoter methylation and transcriptomic changes at the gene and exon levels in developing zebrafish after early life BaP

waterborne exposure, to predict how these changes may lead to immediate, long-term, and multigenerational adverse consequences. The presentation will conclude with how these results contribute to an AOP for BaP-mediated developmental toxicity and risk assessment considerations.

W 1780 Targeted Metabolomics Tools to Inform Systems Biology and Effects-Directed Analysis: Applications in Zebrafish Larvae and Sentinel Species

B. Chandramouli. *AXYS Analytical Services Ltd., Sidney, BC, Canada.*

Assessment of health risks associated with exposure to environmental contaminants currently involves a combination of environmental monitoring and toxicity testing of individual substances. However, environmental monitoring can never be exhaustive, and extrapolating risk from complex substances based on individual exposures is challenging. Targeted metabolomics can be used to characterize unique molecular profiles and aid the identification of affected molecular pathways, enabling adverse outcome pathways evaluation in low dose/sublethal exposures. Furthermore, the changes in absolute concentrations of metabolites can facilitate system biology extrapolations and toxicity predictions across study species and potentially, across studies. Based on quantitative changes of metabolite concentrations, we were able to differentiate the effects of a variety of chemical exposures, including endocrine disruptors, petroleum derivatives, pharmaceuticals and personal by-products, heavy metals and complex environmental samples, on zebrafish (ZF) larvae. Acute exposure to triiodothyronine induced non-monotonic metabolite changes in ZF larvae, linking reduced aminoacyl-tRNA synthesis to reduced amino acid biosynthesis and changes in their metabolism. Endocrine disruptors EE2 and Bisphenol A increased metabolites that are indicative of impacted glycerophospholipid metabolism and steroid hormone biosynthesis and changes in fatty acid biosynthesis. Increases in fatty acids, glycerophospholipid, and sphingomyelins concentrations seen in Triclosan exposed fish suggest increased fatty acid oxidation, altered lipid/energy metabolism and changes in membrane integrity. The ability to profile unique molecular changes in a ZF larvae model exposed to environmentally relevant concentrations of contaminants provides a proof of principle of the validity of quantitative metabolomics in determining functional effects in a developing vertebrate in the absence of morphological endpoints. This effect-directed approach can guide environmental monitoring programs by potentially narrowing the focus of toxicant identification and characterization.

W 1781 Use of Genomic Tools to Determine Novel Adverse Outcome Pathways for the Assessment of Climate Change on Pesticide Toxicity in Salmonid Species of Fish

D. Schlenk. *University of California, Riverside, Riverside, CA.*

Adverse outcome pathways (AOPs) are toxicological tools, which may be used to predict apical endpoints from molecular initiating events (MIEs) for a class of compounds. It is possible that some classes of compounds may have multiple AOPs and/or MIEs. In studies evaluating the impact of hyperosmotic stress resulting from climate change on organophosphate toxicity in salmonids, the classic MIE of acetylcholinesterase inhibition failed to correlate with physiological or whole animal toxicity. In an attempt to understand this, transcriptomic arrays were evaluated in animals after treatment with the individual stressors (hypersaline conditions) and the organophosphate insecticide, chlorpyrifos. Datasets were overlayed and examined for common transcriptional regulatory patterns. Five molecular targets were identified that were consistent with lethal and sublethal physiological and behavioral responses. The hypothesized MIEs focused upon the impairment of neuronal signal transduction primarily through the inhibition of adenyl cyclase activity and alteration of chloride ion channel expression. Impairment of signal transduction was associated physiologically in diminished olfaction and behaviorally in predator-avoidance outcomes. These results demonstrated how transcriptomic responses may be used to identify novel MIEs and potentially develop AOPs for individual or multiple stressor risk assessments.

W 1782 No Genome? No Problem! Development of a High-Quality Computationally Frugal *De Novo* RNA-Seq Method to Enable Predictive Toxicology in Sentinel Species

C. Helbing. *University of Victoria, Victoria, BC, Canada.* Sponsor: S. Huang.

Systems-based approaches provide extensive and unbiased means to determine adverse outcome pathways (AOP) in response to exposure to a chemical of concern or chemical mixtures. These approaches have great potential in augmenting environmental monitoring by enabling evidence-based decision-making and reducing uncertainty regarding biological effects. However, for the vast majority of sentinel species, limited to no genetic information exists thereby severely hampering this approach. This is the case for North American frog species where a major family, the *Ranidae*, is ~300 million year diverged from the African clawed frogs (*Xenopus*) for which genome resources are available. Despite access to *Xenopus* genomes, the degree of nucleic acid sequence divergence is too great to be able to perform standard read alignments. To enable systems-based approaches for the *Ranidae*, we have created a pipeline that permits comparative and computationally frugal *de novo* RNA-seq analysis. Transcriptome Expression and Characterization (TrenCh) works well with popular assembly methods such as Trans-ABySS, IDBA, and Trinity, enhancing their performance substantially by generating ~ 50% longer N50 contig lengths compared to each assembly method alone. We demonstrate the utility of this method through the identification of pathways that are significantly affected in the tissues of the North American bullfrog, *Rana* (Lithobates) *catesbeiana*, upon exposure to disruptors of thyroid hormone action. The same HiSeq data were run on the three platforms with or without TrenCh. Taken together, TrenCh+Trans-ABySS had the greatest overall performance. Application of this method will augment current environmental monitoring efforts in evaluating the potential environmental impact of sensitive non-model or at-risk species in which the genome is unavailable.

W 1783a Scientific and Regulatory Advances in Safety Evaluation of Heavy Metals in Food

B. Mahadevan[1] and M. Krishan[2]. [1]*Regulatory Affairs, Abbott Nutrition, Abbott Laboratories, Columbus, OH and* [2]*ILSI North America, Washington, DC.*

Recent reviews on the risk assessment of contaminants in food, the use of these data in the safety evaluation process, and the safety of the US food supply, in part through its monitoring programs, have led to significant advancements in regulatory and scientific recommendations in the area of food safety. It has also focused on the potential exposure posed by chemicals such as heavy metals, including metals that are essential and may be toxic. In order to prevent potentially unsafe food from reaching the marketplace, regulatory agencies such as the US FDA regulate four major heavy metals (arsenic, lead, cadmium, and mercury) in food. Globally, the Codex Committee on Contaminants in Foods (CCCF) plays a big role in setting safety limits for many of these heavy metals. For example, at the recent CCCF meeting, the maximum levels of lead in the General Standard on Contaminants in food and feed were established. Very few foods are totally free from heavy metals, although they may not be detectable. Therefore, while evaluating food safety from the point of view of heavy metal content, one has to bear in mind that the analysis and detection of heavy metals at the concentration levels commonly found in foods is fairly difficult. However, better analytical and diagnostic procedures have led to the detection of lower levels of heavy metals and how it links to human target organ damage. Most often zero risk/tolerance is expected in risk assessment when confronted with the detection of selected heavy metals in foods/ingredients. In an effort to broaden the understanding of the aforementioned issues pertaining to regulatory toxicology and safety of food, the following key aspects will be addressed in this workshop: 1) Approaches for an efficient method for large-scale surveying of potential toxicities for heavy metal contaminants; 2) global regulatory programs that help with enforcement and setting of safe exposure limits; 3) availability of a heavy metal dietary exposure screening tool for rapid evaluation of potential public health risk; and 4) applicability of such a tool to support efficient food safety risk management practices.

1783b Heavy Metals in ToxCast: Relevance to Food Safety

K. Houck. *ORD/NCCT, US EPA, Research Triangle Park, NC.*

Human exposure to heavy metals occurs through food contamination due to industrial processes, vehicle emissions and farming methods. Specific toxicity endpoints have been associated with metal exposures, e.g. lead and neurotoxicity; however, numerous varieties of heavy metals have not been systematically examined for potential toxicities. We describe results from testing a large set of heavy metal-containing compounds in extensive suites of *in vitro* assays to suggest possible molecular initiating events in toxicity pathways. A broad definition of heavy metals that includes As, Se and organometallics or inorganic salts containing metals in Group III or higher (MW > 40) was used to identify 75 different compounds tested in the EPA's ToxCast assays encompassing biochemical, cellular and model organism assays. These 75, plus an additional 100 metal-containing compounds, were tested in Tox21 quantitative high-throughput screening (qHTS) assays covering nuclear receptor and stress pathways. Known activities were confirmed such as activation of stress pathways and nuclear receptors (RXR, PPARg) as well as overt cytotoxicity. Specifically, organotin and organomercury were among the most potent of over 8K chemicals tested. The HTS results support known toxicities, including promiscuous GPCR activity for mercury compounds consistent with the neuropsychiatric effects seen in mercury poisoning (Mad Hatter's Syndrome). As such, HTS approaches provide an efficient method for surveying potential toxicities of heavy metals and suggest areas of focus to more thoroughly examine toxicity links and define safe exposure limits. These data may be of use to government and industry safety assessors as well as academics and others focused on mechanism of action. [This abstract does not necessarily reflect US EPA policy]

1784 FDA and Codex Activity on Heavy Metals in Food

L. P. Robin. *DPPB/OFS/CFSAN US FDA, College Park, MD.* Sponsor: B. Mahadevan.

The talk will discuss how the Food and Drug Administration (FDA) regulates heavy metals, such as arsenic, lead, cadmium, and mercury, in food. Topics will include FDA's Toxic Elements Program, FDA's Total Diet Study Program, FDA statutory authority and enforcement options, and recent regulatory developments on heavy metals. The talk will also cover recent activities on heavy metals at the Codex Committee on Contaminants in Food, including the US-led initiative to lower maximum levels for lead in food in Codex's General Standard for Contaminants and Toxins in Food and Feed. Topics covered should be relevant to those interested in regulation of heavy metals in food both domestically and internationally, such as representatives of government agencies, academia, and industry.

1785 Introduction to the Metal Dietary Exposure Screening Tool

N. Tran. *Exponent Inc., Washington, DC.*

A Metal Dietary Exposure Screening Tool (MDEST) was developed to address the need for rapid evaluation of the potential public health risks associated with the frequent detection of heavy metals at trace or higher levels in foods and food ingredients. Food intake rates based on the National Health and Nutrition Examination Survey (NHANES) 2005–2010 consumption data for the United States (US) population 2+ years (yrs) and for infants age 6 months to <2 yrs based on the Feeding Infants and Toddlers Study (FITS), existing exposure limits for inorganic arsenic, cadmium, chromium, lead, and mercury, were incorporated in the MDEST. The tool has data entry fields for detected concentrations and includes algorithms that combine metal levels with consumption data to generate screening level exposure estimates, which are then compared to the appropriate exposure limits in the risk characterization module. As a screening level tool, the risk assessment output is intentionally conservative and public health protective, intended for a rapid assessment to set aside issues that are not of concern, and to identify those needing further evaluation with refined data input tailored to the specific situation under consideration.

1786 Case Studies Demonstrating the Utility of the Metal Dietary Exposure Screening Tool

C. Llewellyn. *The Coca-Cola Company, Atlanta, GA.*

Metals are ubiquitous in the environment and can be found in many foods and food ingredients. As the capabilities of analytical tools have progressed, greater numbers of findings at lower and lower levels of metals in foods and ingredients is occurring. Metals are physiologically active with some be beneficial but exposures to others can present potential risks associated with long term consumption to consumers. Guidance levels for tolerable intake for numerous metals are being lowered or being removed by food safety organizations. Because metals can often be found in foods, food safety professionals are faced with decisions on analytical findings of metals in foods and food ingredients. A consistent approach is needed to evaluate how such findings in specific foods or food ingredients can be evaluated in a consistent and conservative fashion. The Metal Dietary Exposure Screening Tool (MDEST) was developed to be a resource that provides a conservative and consistent approach to help address and prioritize analytical findings for the metals; inorganic Arsenic, Cadmium, Chromium III and VI, Lead, and Mercury. The MEDST was developed to evaluate the contribution of a single food product type to a developed or existing guidance level for tolerable intake of the 6 metals included in the tool. This approach provides a conservative estimate of the contribution that a single food to the total intake of the 5 metals. The background contribution of other foods and drinking water to the calculated or referenced tolerable intake levels was calculated for each of the 6 metals. The portion of the tolerable intake available for the food type of interest is determined using the dose of metal contained in a serving of a food, and based on the particular food's contribution to the total diet using NHANES data from 2008-2010. The utility of the MDEST will be demonstrated using 2 case studies. The case studies will demonstrate the use of the tool to assist in decision making for analytical findings for 2 metals in a food and a food ingredient.

1787 Risk Assessment of Metals in Food Utilizing Mode of Action Analysis

S. M. Cohen. *University of Nebraska Medical Center, Omaha, NE.*

Several metals are present in food and water, some essential (e.g. Mg, Mn, Fe, Cu) and some potentially toxic (e.g. As, Cr, Ni). Evaluation of the risk of these metals involves similar issues, including oxidative state, inorganic vs. organic forms, exposure, metabolism, and mode of action. Addressing these issues is critical for academic researchers and for those in industry and regulatory agencies addressing risk assessment. Arsenic, as a typical example, is present in various forms in food and drinking water and is known to be associated with various cancer and non-cancer toxicities. Exposure analysis is dependent on identification of the forms of arsenic present in the diet and drinking water. Inorganic arsenic, the dominant toxic form we are exposed to, is toxic, whereas many of the organic arsenicals are much less toxic or innocuous. The mode of action of inorganic arsenic toxicity and carcinogenicity is dependent on an understanding of the metabolism, which proceeds through a sequence of reductions followed by oxidative methylations. The trivalent forms of arsenic are highly reactive with protein sulfhydryl groups, but are not reactive with DNA. Depending on the specific protein with which it interacts, various biological effects are produced. Many of these effects are related to cytotoxicity with consequent regeneration. In epithelial systems, this can result in cancer. It appears that in *in vitro* and *in vivo* models and in epidemiologic evaluations, arsenic toxicity and carcinogenicity is a threshold phenomenon, as would be expected based on its interaction with proteins rather than DNA.

1788 Reproducibility of Results in the Zebrafish Embryotoxicity Assay

B. Flick, J. Hemmerich, E. Salinas, T. Ramirez, B. van Ravenzwaay and J. Otte. *Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

The zebrafish model is established in various laboratories to predict developmental toxicity, but there is no protocol providing detailed information morphological features (scores) to be assessed nor about the reproducibility of score results. In this context, we aimed to optimize morphological scoring and reproducibility. A blinded study was performed using 4 test substances (A - triazole fungicide, B - pyradizine amide insecticide C pyridine amine fungicide, D - triazole fungicide) with 3 trained experimenters in 3 independently repeated experiments. Test substances covered a broad range of developmental toxicity *in vivo*: (A) no toxicity, (B) and (C) maternal and developmental toxicity at the same dose level and (D) teratogenic effects without no maternal toxicity.

Zebrafish were exposed to 7 concentrations over 5 days post fertilization and evaluated by 53 endpoints. The resulted data set was analyzed for reproducibility within and between the experimenters. Overall accuracy of prediction was 75% varying from 44% to 100% between the experimenters. The total reproducibility of the prediction based on 1 experiment was 60% and varied from 0 to 100% between the experimenters. On average 32% of 53 morphological endpoints were detected as altered by the 4 test substances. 50% of those were observed only by one, 27% by two and only 22% by all 3 experimenters. In this study the test substance concentrations were also unknown to the experimenters. Concentration-response relationship was weak resulting in poor EC50 values with a large variation between experimenters. Following this analysis morphological score was simplified to get more reproducible results. Endpoints were reduced in more specific but less detailed endpoints (from 53 down to 31). Simplification was achieved by summarizing former specific different phenotypes of one organ anlage or linked embryonic developmental processes to broader endpoints. On paper this simplification leads to more reproducible results with an improved accuracy.

PL 1789 Comparison of Predictivity for Developmental Toxicity in 3 Zebrafish Strains

W. Nowland, R. St. Louis, C. Stethem and D. Stedman. *Developmental and Reporductive Toxicology, Pfizer Inc., Groton, CT*. Sponsor: G. Cappon.

Zebrafish (ZF) have become a valuable tool in screening for toxicity and developmental malformations, with some suggesting that ZF could evolve into a suitable replacement for one or both of the mammalian species typically used for evaluation of developmental toxicity. Revisions of ICH S5(R2) to include using all available data as a weight of evidence approach to reduce the number of embryo fetal development studies suggest the implementation of alternative assays may result in the reduction of animal studies. The Zebrafish Development Toxicity Assay (ZDTA) assay exposes ZF embryos to the test compound continuously from 6-120 hours post-fertilization (hpf), at which time the ZF are evaluated for morphological development. One hurdle to widespread adoption of ZDTA is inter-laboratory reproducibility. As there are regional differences in ZF strains used in the ZDTA, strain-specific differences in susceptibility to developmental toxicants could have a meaningful impact on inter-laboratory reproducibility. We recently investigated and compared results from ZDTA using three strains of ZF. Pfizer Wild Type (WT), Tuebingen (TU) and NHGRI-1 zebrafish strains were assessed against 11 small molecule pharmaceutical compounds with completed rat and/or rabbit developmental toxicity studies. The morphological scores of each compound in each strain were collected and the no observed effect concentration (NOEC), lowest observed effect concentration (LOEC) for altered phenotypes along with the lowest dose at which death occurred in all fish (LC100) were compared across strains. Additionally, teratogenicity was evaluated using three published developmental toxicology algorithms. The prediction for developmental toxicity, as well the NOEC, LOEC and LC100 values were similar in all 3 strains tested suggesting that the ZF strain used in the ZDTA may not be a significant source of variation.

PL 1790 Targeted Proteomic Analysis Associated with an *In Vitro* Zebrafish Developmental Toxicology Assay Using LC-MS/MS

J. C. Olavarria[2], A. Machin[2], K. Apgar[2], C. Fernandez-Metzler[1] and R. King[1]. [1]*Pharmacadence Analytical Services, Hatfield, PA and* [2]*Toxicology, Lampire Biological Laboratories, Pipersville, PA.* Sponsor: J. Hillegass.

Opportunities exist to incorporate *in vitro* developmental toxicology studies early into the drug discovery process as a means of proactively identifying teratogenic compounds. One of the most promising assays utilizes zebrafish embryos, which due to their *ex vivo*/rapid development, transparency, and relatively good conservation of embryological processes, represent a model organism for developmental toxicology studies. The zebrafish embryo culture (ZEC) assay calls for the exposure of 4-6 hour post fertilization embryos to 0.1, 1, 10, and 100 µM of the test article of interest, and subsequent assessment of viability and morphology at 5 days post fertilization (dpf). While this data allows for determination of teratogenic liability in a high-throughput manner, further determination of mechanism of compound action is seldom made. To this end, the use of LC-MS/MS and targeted proteomic analysis provides a powerful platform for the identification and quantitation of all the proteins within a specific biological pathway from a single sample. In a proof of concept study, the standard ZEC assay was employed to assess the teratogenicity of a series of glucocorticoids, including dexamethasone and hydrocortisone. Since these compounds are known to induce matrix metalloproteinase (MMP) expression in developing zebrafish embryos, glucocorticoid-treated embryo lysate samples were prepared at 5 dpf following morphology scoring procedures and submitted for quantification of relative protein amounts within a matrix remodeling pathway. Results indicate that targeted proteomic analysis via LC-MS/MS as a follow up to the standard ZEC assay provides a highly sensitive means of protein quantification and pathway analysis, and could potentially serve as a "first-line" indication of mechanism of action for pharmacological compounds.

PL 1791 Microbiota Colonization Status Influences Developmental Toxicity of Bisphenol A in Embryonic Zebrafish

T. Tal[3], D. Betancourt[3], D. Hunter[3], S. Espenschied[2], J. Rawls[2], S. Charla[3], T. Dean[3] and C. Wood[3]. [1]*Air Pollution Prevention and Control Division, US EPA, Research Triangle Park, NC;* [2]*Department of Molecular Genetics and Microbiology, Duke University, Durham, NC and* [3]*Integrated Systems Toxicology Division, US EPA, Research Triangle Park, NC.*

There is growing evidence that microbiota can modify the toxicokinetics and/or toxicodynamics of environmental chemicals. Commonly used mammalian systems have limited ability to link phenotypic effects in exposed animals to colonization status. Here, we used gnotobiotic zebrafish to explore whether chemical-induced developmental toxicity outcomes are sensitive to colonization status. Gnotobiotic zebrafish were generated at 0 days post fertilization (dpf). At 1 dpf, culture sterility was assessed and a subset of gnotobiotic zebrafish were colonized with complex microbiota harvested from a conventional zebrafish aquaculture facility. Gnotobiotic and conventionalized cohorts were statically exposed to 1, 10, 40, or 100 µM bisphenol A (BPA) or 0.4% DMSO from 1-6 dpf. Culture sterility, developmental toxicity, and neurobehavioral development using a standard locomotor assay consisting of alternating light and dark periods, were assessed at 6 dpf. No differences in overt developmental toxicity were observed among BPA-exposed gnotobiotic and conventionalized zebrafish. Significant mortality occurred at > 40 µM BPA, regardless of the presence or absence of microbiota. With regard to neurobehavioral development, there were no differences in mean movement during light or dark periods in gnotobiotic and conventionalized vehicle control larvae. Significant hypoactivity in the dark epoch was observed in gnotobiotic and conventionalized zebrafish exposed to 10 µM BPA relative to vehicle controls. A significant interaction between colonization status and exposure group was also noted, reflecting hyperactivity in gnotobiotic versus conventionalized zebrafish exposed to 1 µM BPA. These data show that normal microbiota are capable of shifting the chemical potency of BPA during development. This abstract does not necessarily reflect US EPA policy.

PL 1792 Embryonic Atrazine Exposure Elicits Alterations in Genes Associated with Neuroendocrine Function in Adult Male Zebrafish

S. E. Wirbisky[3], M. S. Sepulveda[3], G. J. Weber[3], A. S. Jannasch[3] and J. L. Freeman[3]. [1]*Purdue University, West Lafayette, IN;* [2]*Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN and* [3]*School of Health Sciences, Purdue University, West Lafayette, IN.*

The developmental origins of health and adult disease (DOHaD) hypothesis states that exposure to environmental stressors early in life can elicit changes to the genome and epigenome thereby resulting in an increased susceptibility of a disease state during adulthood. Atrazine, a common herbicide used throughout the Midwestern United States for control of weeds in agricultural crops contaminates potable water supplies and is a suspected endocrine disrupting chemical. Studies suggest atrazine elicits reproductive dysfunction through the hypothalamus-pituitary-gonadal axis. In previous studies, zebrafish were exposed to 0.3, 3, or 30 parts per billion (ppb; µg/L) atrazine through embryogenesis, rinsed, and allowed to mature to adulthood in clean water. Results showed a decrease in spawning and an increase in progesterone and ovarian follicular atresia in adult females. In addition, transcriptome alterations in brain and ovarian tissue support neuroendocrine dysfunction. As reproductive dysfunction may also be influenced by males, this study assessed testes morphology and histology, hormone levels, and transcriptomic profiles of testes and brain tissue in adult males exposed to atrazine during embryogenesis. The embryonic atrazine exposure resulted in no alterations in body or testes weight, gonadosomatic index, testes histology, or levels of 11-ketotestosterone or testosterone. Transcriptomic profiles of testes tissue demonstrated alterations in genes associated with proper lipid and steroid function. In addition, transcriptomic profiles of adult male brains revealed alterations in

genes associated with cellular signaling and molecular transport. Thus, although the transcriptomics data indicate later-in-life alterations on the neuroendocrine system of adult males exposed to atrazine during embryogenesis, analysis of additional endpoints is needed to determine functional impairments.

1793 Acute Developmental Toxicity of Crude MCHM in Zebrafish (*Danio rerio*) and Observed Neurobehavioral Alterations

K. A. Horzmann[2], A. J. Whelton[2] and J. L. Freeman[2]. [1]*Environmental and Ecological Engineering, Purdue University, West Lafayette, IN and* [2]*Health Sciences, Purdue University, West Lafayette, IN.*

On January 9, 2014, a state of emergency was declared in Charleston, West Virginia after an estimated 37,854 liters of crude 4-methycyclohexane methanol (MCHM) leaked from a storage tank into the Elk River just 2.4 km upstream of a water treatment plant that provided water to approximately 15% of West Virginia's population. The environmental and public health implications of this chemical spill are still being assessed, as MCHM was grandfathered in through the Toxic Substances Control Act of 1976. In this study, the zebrafish model was first used to evaluate acute developmental toxicity by monitoring mortality and hatching through 120 hours post fertilization (hpf). Zebrafish embryos were dosed immediately after fertilization with 0, 1, 6.25, 12.5, 25, 50, or 100 parts per million (ppm; mg/L) crude MCHM. The 100 ppm treatment group experienced 100% mortality by 30 hpf, while there was no significant difference in survivability between the other treatment groups ($p>0.05$). The 50 ppm treatment group had significantly decreased percent hatched at 54 hpf ($p<0.05$). A photomotor response test performed at 120 hpf showed significantly decreased total distance moved and decreased velocity compared to controls at 1, 12.5, and 50 ppm dose levels ($p<0.05$). This study suggests that, based on the results of the photomotor test, the neurotoxicity of crude MCHM may be greater than previously reported.

1794 Identification of Skeletal Disruptor Compounds Through *In Vivo* Screening in Zebrafish

S. Thrikawala[2], R. Sachanandani[2], J.-k. Gustafsson[2] and M. Bondesson[2]. [1]*Department of Biology and Biochemistry, Center for Nuclear Receptors and Cell Signaling, University of Houston, Houston, TX and* [2]*Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX.* Sponsor: D. Zalko.

Exposure to teratogens during embryonic development may lead to congenital skeletal birth defects, including cranio-facial and limb malformations. We have used zebrafish as a model organism to identify skeletal disruptors. Transgenic zebrafish expressing fluorescent markers in bone or cartilage tissues were used to visually monitor the development of the cleithrum, craniofacial cartilage structures and the notochord under normal and perturbed conditions. Next, transgenic skeletal embryos were used in a primary high-throughput screen of 301 compounds of the ToxCast phase I chemical inventory, primarily consisting of pesticides and antimicrobials. We found that exposure to 40 compounds perturbed normal development of the cleithrum and 13 compounds caused a notochord effect. Ten compounds perturbed both cleithrum and the notochord. The compounds causing an effect in the primary screen were further analyzed across a wider dose range in both the bone and cartilage transgenic fish. In conclusion, our results show that several environmental pollutants have skeletal disrupting capacity and that the developing zebrafish embryo is an efficient *in vivo* model that can be used for identification of skeletal disruptors.

1795 Strain-Specific Changes in Locomotor Behavior in Larval Zebrafish Elicited by Cholinergic Challenge

J. M. Hedge, K. A. Jarema, D. L. Hunter and S. Padilla. *Integrated Systems Toxicology Division, NHEERL, ORD, US EPA, Research Triangle Park, NC.* Sponsor: C. McQueen.

Some studies have compared the baseline behavior of different strains of larval zebrafish (*Danio rerio*), but there is sparse information on strain-specific responses to chemical challenges. The following study examines both the basal activity and response to a pharmacological challenge in five zebrafish strains: Streisinger AB (AB), Wild India Karyotype (WIK), Tupfel long fin (TL), Sanger AB Tübingen (SAT) and our in-house wild type, out-bred strain (Z); all strains, excepting our own, were obtained from Zebrafish International Resource Center (Eugene, OR). On day 0, zebrafish embryos of each strain were plated in one of two 96-well plates (n=16/strain/plate). At 6 days post fertilization (dpf) we examined both basal locomotor activity and activity after an acute chlorpyrifos (CPF; 11.5μM) challenge in a light:dark test paradigm. CPF is a pesticide that is activated by the liver to a potent cholinesterase inhibitor. The testing paradigm consisted of dosing with chlorpyrifos, then waiting for 30 minutes followed by a 6 minute basal period in the dark, 10 minutes of light and 10 minutes of dark. During the dark periods, the controls (DMSO vehicle treated) showed marked differences in activity, with the TL strain the most active and the WIK the least active; in the light period, the controls all showed comparable activity. All strains showed hyperactivity in response to the CPF challenge, indicating that all were capable of hepatic activation of the CPF and responsive to the behavioral disruption, but there were differences in the level of activity among the strains--most notable during the basal dark period and the light period. These results indicate strain type can influence baseline activity and also activity in response to a drug challenge, and that these interactions are dependent on the light level during the testing, making strain choice an important consideration in research planning. (This abstract may not necessarily reflect official Agency policy)

1796 Defining the Developmental Toxicity of Commonly Used Flame Retardant Chemicals Using Zebrafish

S. Mann[2], L. Truong[1], G. Gonnerman[1] and R. Tanguay[1]. [1]*Oregon State University, Corvallis, OR and* [2]*University of California, Davis, Davis, CA.* Sponsor: R. Tanguay.

Increased fire safety standards and the usage of flammable plastics and electronics have resulted in the heightened use of flame retardant chemicals in consumer products. The uncertainty of the safety of such flame retardants has led to the study of zebrafish as a nonmammalian model for flame retardant chemical toxicity testing. Zebrafish have proven to be a useful model for screening chemicals for human health hazard and for disease related research because they are easy to manipulate genetically and pharmacologically. In this study, dechorionated zebrafish embryos were exposed to nine flame retardant chemicals from 6 to 120 hours post fertilization. Chemical addition occurred at five different time points in development to measure potential differences in susceptibility to toxicity at six concentrations. Behavioral responses were also measured using embryonic and larval photomotor response assays. Developmental effects were evaluated at 24 and 120 hpf to determine whether the time of chemical addition impacted developmental toxicity. Based on the analysis of the developmental end points, 4/9 flame retardants tested showed that the developmental time of chemical exposure influenced the toxicity of that chemical, while the remaining 5 chemicals were not influenced by the developmental time of chemical exposure. Therefore, these results indicate that there are clear differences in the developmental toxicity of flame retardant chemicals and for some of these chemicals there are narrow developmental windows of susceptibility. Future research needs include testing additional flame retardants for stage specific toxicity and determining the amount of chemical taken up by the zebrafish during exposure in order to further analyze the impact and safety of flame retardant chemicals.

1797 Transcriptome and DNA Methylome Profiling of Early Zebrafish Embryos Exposed to TDCPP

D. C. Volz[2], J. Leet[3], D. Altomare[3], A. Chen[1], H. Stapleton[1], Y. Yu[2] and Y. Wang[2]. [1]*Duke University, Durham, NC;* [2]*University of California, Riverside, CA and* [3]*University of South Carolina, Columbia, SC.*

Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) is a high-production volume organophosphate-based flame retardant (OPFR) widely used as a PentaBDE replacement. Using zebrafish as a model, we previously demonstrated that the cleavage period (0.75-2.25 hours post-fertilization, hpf) during embryogenesis is susceptible to TDCPP-induced delays in methylation of the zygotic genome. Therefore, the objective of this study was to determine whether (1) TDCPP is genotoxic within human lymphoblastoid TK6 cells, as previous reports suggest that TDCPP may be mutagenic; (2) TDCPP inhibits zebrafish DNA methyltransferase (DNMT) within embryonic nuclear extracts; and (3) uptake of TDCPP from 0.75 hpf (2-cell) to 2 hpf (64-cell) or 6 hpf (shield stage) leads to impacts on the early embryonic transcriptome and DNA methylome. For DNMT activity assays and all embryonic exposures, 5-azacytidine (5-azaC, a reference DNMT inhibitor) was used as a positive control. TDCPP was negative for genotoxicity at all non-cytotoxic concentrations tested (1.95-31.25 μM) and, although 5-azaC significantly inhibited zebrafish DNMT, TDCPP did not affect DNMT activity at concentrations as high as 500 μM. Moreover, despite significant uptake of TDCPP as early as 2 hpf,

total 5-methyl-2′-deoxycytidine (5-mdC) levels were not different within 2- and 6-hpf embryos exposed to maximum tolerated concentrations of 5-azaC (250 µM) or TDCPP (2 µM). However, 5-azaC and TDCPP exposure resulted in chemical-specific alterations of the embryonic transcriptome at 6 hpf (but not 2 hpf), suggesting that (1) 5-azaC- and TDCPP-induced effects on transcription were not due to differential DNA methylation or (2) we were unable to detect subtle differences in 5-mdC within embryonic gDNA. Therefore, to address both potential outcomes at single base-pair resolution, we are conducting whole-genome bisulfite sequencing on Illumina's NextSeq 500 platform to identify the magnitude and extent, if any, of 5-azaC- and TDCPP-induced effects on the DNA methylome at 2 or 6 hpf.

1798 Triclosan Is a Mitochondrial Uncoupler in Live Zebrafish

J. Shim[2], L. M. Weatherly[2], R. H. Luc[2], M. T. Dorman[2], A. Neilson[1], R. Ng[1], C. H. Kim[2], P. J. Millard[2] and J. A. Gosse[2]. *[1]Seahorse Bioscience, Billerica, MA and [2]University of Maine, Orono, ME.*

Triclosan (TCS) is a synthetic antimicrobial agent used in many consumer goods at millimolar concentrations. As a result of exposure, TCS has been detected widely in humans. We have recently discovered that TCS is a proton ionophore mitochondrial uncoupler in multiple types of living cells. Here we present novel data indicating that TCS is also a mitochondrial uncoupler in a living organism: 24 hour post fertilization zebrafish embryos. These experiments were conducted using a Seahorse Bioscience XFe 96 Extracellular Flux Analyzer modified for bidirectional temperature control, using the XF96 spheroid plate to position and measure one zebrafish embryo per well. Using this method, following acute exposure to TCS, basal oxygen consumption rate (OCR) increases, without a decrease in survival or heart beat rate. TCS also decreases ATP-linked respiration and spare respiratory capacity and increases proton leak: all indicators of mitochondrial uncoupling. Our data indicate, for the first time, that TCS is a mitochondrial uncoupler *in vivo*, which should be taken into consideration when assessing the potential toxicity and/or pharmaceutical uses of TCS. To our knowledge, this is the first example of the Seahorse 96-well Extracellular Flux Analyzer being used to measure bioenergetic flux of a single embryo per well in a 96 well assay format. The method developed in this study provides a novel high-throughput tool to identify previously-unknown mitochondrial uncouplers in a living organism.

1799 Characterization of a Pxr-null Zebrafish

M. C. Salanga[2], A. Kubota[2], B. Lemaire[2], R. Harbeitner[2], D. Gusenleitner[1], S. Monti[1], J. J. Stegeman[2] and J. V. Goldstone[2]. *[1]Boston University, Boston, MA and [2]Biology, Woods Hole Oceanographic Institution, Woods Hole, MA.*

The pregnane X receptor (PXR) (nuclear receptor NR1I2) is a ligand activated transcription factor, mediating responses to diverse xenobiotic and endogenous chemicals. The properties of PXR in fish are not fully understood. Teleost fish lack CAR (NR1I3), a closely related receptor in tetrapods that is involved in the response to many xenobiotics, magnifying the potential role of PXR in fish. *In vitro* studies using isolated ligand binding domains (LBDs) in COS cells have indicated differences in ligand binding between human and zebrafish PXRs. However, these *in vitro* methods do not adequately reflect the complexity of transcription factor activation *in vivo*, where full length protein occurs in the normal cell context. Here we report on pxr expression *in vivo*, including transcriptome analysis by RNA-Seq, regulation of pxr and the Pxr target gene cyp3a65 by human PXR agonists, and the generation of a pxr knockout strain. A pxr-null zebrafish strain was generated the CRISPR-Cas9 genomic editing method. Three separate sgRNA were targeted to exon 2 of AB strain embryos and co-injected with Cas9 protein, introducing multiple lesions. Injected embryos were raised, outcrossed to produce heterozygous F1, and siblings were backcrossed to create homozygous mutants. The combination of pregnenolone dosing and morpholino knock-down of pxr was previously used to define the Pxr gene battery in zebrafish, and compared to transcriptomic analyses of pxr knockout zebrafish. Differentially regulated genes following morpholino knockdown included pxr, cyp2k6, cyp3a65, and abcb5. The prototypical human and mouse PXR agonist hyperforin downregulated the expression of both pxr and cyp3a65, with an IC50 less than 500 nM. Other human PXR agonists downregulated cyp3a65 but not pxrexpression, including nifedipine and triclosan, but not pregnenolone sulfate. The description of the Pxr gene battery and the generation of a knockout strain in zebrafish has implications for assessing the action of PXR ligands in fish, including many pollutants. (NIH P42ES007381 (BU SRP), NIH R21HD073805).

1800 Elucidating Gene by Environment Interactions (GxE) Associated with Interindividual Variation in Response to Chemical Exposure

M. Meisner[1], L. Truong[2], R. L. Tanguay[2] and D. M. Reif[1]. *[1]Bioinformatics Research Center, North Carolina State University, Raleigh, NC and [2]Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR.*

There are limited, and often conflicting, data on how the effects of environmental agents differ across susceptible populations. We hypothesize that gene-environment interactions (GxE) play an important role and can be detected using a zebrafish system that allows comprehensive analysis of phenotypic endpoints, genetic factors, and environmental exposures. First, we exploited the large-scale design of a high throughput screening (HTS) system that has tested thousands of chemicals in an outbred zebrafish population of unknown genetic heterogeneity to select Abamectin as a chemical that displayed characteristic phenotypic patterns and high variability between individual responses. We characterized the correlation structure across morphological endpoints in order to describe a multivariate phenotype of altered eye, snout, jaw, pericardial edema, yolk sac edema, and axis development in zebrafish exposed to Abamectin. Second, we performed range-finding studies to narrow our estimate of the Abamectin concentration to the critical concentration at which a stable 50:50 affected:unaffected proportion was observed. Third, samples were exposed to the critical [0.6 uM] concentration of Abamectin. At 5 days post-fertilization (dpf), we isolated "affected" samples that displayed our multivariate phenotype and "unaffected" samples that did not respond to chemical exposure. DNA was then individually isolated and sequenced from each affected and unaffected sample. These data were then used to identify GxE as genetic variants associated with interindividual variation in response to chemical exposure. The approach, wherein we exert fine-scale control of the exposure environment and obtain reliable phenotypic assessments, can be extended to new chemicals or other types of environmental exposures and will hopefully shed light on genetic regions for targeted follow-up.

1801 Genomic Editing with Crispr/Cas9 Confirms Pharmaceutical Target Knockdown Phenotype in Zebrafish

D. B. Stedman, S. W. Kumpf, W. S. Nowland, C. M. Stethem, R. St. Louis, C. J. Bowman and G. D. Cappon. *DSRD, Pfizer, Groton, CT.*

Many current approaches to implementing the 3 R's include using all available data in a weight of evidence approach to reduce the number embryo-fetal development studies. In this report, we describe an approach for the use of Zebrafish as an alternative model for teratogenicity assessment of the Smoothened inhibitor (SMOi) PF-04449913 (-913) and by genomic editing. SMO is a key developmental gene, and its inhibition by SMOi's like cyclopamine leads to profound developmental toxicity in mammals. Both cyclopamine and -913 were tested in a Zebrafish Development Toxicity Assay, where zebrafish embryos were exposed continuously to test compounds starting at 6 hours post fertilization (hpf). At approximately 120 hpf cultures are terminated and the larvae evaluated for abnormal phenotypes. Both cyclopamine and -913 produced dose-dependent malformations with a profound distal axial U-shape body curve. To confirm that this dysmorphogenesis was due to inhibition of smoothened signaling, we generated Zebrafish SMO-specific mutants using Clustered, Regularly Interspaced, Short Palindromic Repeat (CRISPR)/Cas9 technology to selectively mutate the SMO gene in Zebrafish and compare the phenotype to that seen after cyclopamine or -913. Specific single-guide RNAs (sgRNAs) targeting the coding region of SMO, and Cas9 protein/RNA were co-injected into single-cell-stage Zebrafish embryos. Phenotype evaluation, cloning and sequencing of defined PCR products, were used to confirm targeted gene disruptions. Importantly, CRISPR/Cas9 SMO mutant phenotypes were remarkably similar to those seen with both cyclopamine and -913 treatments. Injecting a combination of four SMO sgRNA's give a phenotype efficiency >90%. PCR was performed using flanking primers on pooled CRISPR/Cas9 injected embryos followed by cloning and individual sequencing of the representative PCR products. Sanger sequencing confirmed various mutation types (insertions and deletions) mainly orchestrated by two of the four SMO sgRNAs. This work confirmed that the terata produced by -913 were due to SMO inhibition.

1802 Phenotypically-Anchored Transcriptomic Response in Embryonic Zebrafish Developmentally Exposed to the Antimicrobial Agent, Triclosan

D. E. Haggard[2], P. D. Noyes[2], F. Tilton[2], D. Thomas[1], K. M. Waters[1] and R. L. Tanguay[2]. [1]*Pacific Northwest National Laboratory, Richland, WA and* [2]*Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Triclosan (TCS) is an antimicrobial agent found in many personal care products including hand soaps, shampoos, and toothpastes. Due to its widespread use, TCS also readily enters the environment through wastewater and has been detected in human plasma, urine, and breast milk. Furthermore, TCS disrupts both estrogen and thyroid hormone signaling in several *in vivo* model systems; therefore, research examining the developmental effects of TCS exposure is warranted. Here, we utilized phenotypically-anchored whole genome transcriptomics to investigate the developmental toxicity and potential mechanism of action of TCS in embryonic zebrafish. Dechorionated zebrafish embryos were exposed to graded concentration of TCS from 6-120 hours post fertilization (hpf) and mortality and morbidity were assessed at 24 and 120 hpf. Exposure to 8 µM or higher lead to mortality at 120 hpf with a calculated EC80 of 7.36 µM. Embryos were subsequently exposed to the EC80 concentration from 6-48 hpf after which total RNA was extracted and full genome transcriptomic analysis was performed using high-density microarrays. We identified a total of 518 significantly elevated and 1972 significantly reduced transcripts compared to control (FDR adjusted p-value ≤ 0.05; fold change ≥ 1.5). Principal component analysis showed good separation between the DMSO control and TCS exposure groups. Pathway and gene ontology enrichment analyses of the significant differentially expressed transcripts using MetaCore and the DAVID annotation tool revealed enrichment in pathways and biological processes involved in synaptic transmission, immune response, glycolysis and gluconeogenesis, and nervous system development. These studies indicate that TCS is highly bioactive *in vivo*; consistent with the diverse *in vitro* activity reported in the ToxCast dashboard. This research was supported by EPA #R835168, NIH P30 ES000210, and T32 ES007060.

1803 Toxicity Screening of a Subset of the ToxCast Phase III Chemical Library Using a Zebrafish Developmental Assay

K. Houck[2], S. Mosher[1], R. Judson[2], J. Wambaugh[2], M. Martin[2] and S. Padilla[2]. [1]*NCCT, ORISE, Research Triangle Park, NC and* [2]*NCCT, US EPA, Research Triangle Park, NC;* [3]*NHEERL, US EPA, Research Triangle Park, NC.*

As part of the ToxCast chemical screening and prioritization research program of the US EPA, 500 ToxCast Phase III chemicals were assessed using a vertebrate screen for developmental toxicity. An initial triage was conducted of the full 1000 Phase III chemicals using a mammalian cellular transcription factor activation assay. The 500 most active were then tested in the Zebrafish (*Danio rerio*) development assay. Larvae were exposed in 96-well plates from late-blastula stage (6hr post fertilization, pf) through day 5pf (1–2 days post-hatch). All exposures were renewed daily. Intra- and inter-plate replicates were included for quality control. Developmental toxicity was first assessed using a single nominal concentration of ≈ 80 µM. On day 5pf, larvae were moved from exposure solution to a control solution without chemical, and on day 6pf were assessed for overt toxicity (i.e., death, non-hatching and dysmorphology; n=4 embryos per chemical). Dysmorphology was a combined score using both in-life observation and Brightfield, high-content image analysis. Results showed 333 chemicals positive at the single concentration and 167 negative, with 75% concordance among the 8 inter-plate controls. The mammalian assay pre-screening resulted in a higher incidence of positives then in previous chemical sets with overt toxicity noted with 67% of the chemicals tested, compared to previous studies yielding 40% positives with Phase II and 62% positive chemicals in the ToxCast Phase I library (consisting of mostly pesticide active ingredients). The use of high-throughput screening techniques including automated imaging analysis provides the means to perform highly complex biological *in vivo* assays covering critical pathways of development for large numbers of important chemicals. This abstract may not necessarily reflect official Agency policy.

1804 A Novel Orthotopic Embryo-Larval Zebrafish Xenograft Assay to Assess New Glioblastoma Therapeutics Efficacy

L. C. Wehmas[2], R. L. Tanguay[2] and J. A. Greenwood[2]. [1]*Biochemistry & Biophysics, Oregon State University, Corvallis, OR and* [2]*Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR.*

Despite decades of research, glioblastoma remains an aggressive brain cancer with limited treatment options and dismal survival rates (~12 months). Discovering new compounds that mitigate glioblastoma progression is vital to improving patient survival. We have developed a four day orthotopic embryo-larval zebrafish xenograft assay to identify and prioritize compounds that impact human glioblastoma proliferation, migration and invasion. We illustrate the utility of our assay by testing the well-studied, phosphatidylinositide 3-kinase inhibitor LY294002. While exposure to the highest concentration of LY294002 (12.5 µM) caused significant mortality (~49%), exposures to 3.125-6.25 µM LY294002 caused little to no adverse effects and significantly decreased human glioblastoma proliferation by up to 34% compared to vehicle control. The effects on proliferation trended toward concentration dependence. Furthermore, exposure to 6.25 µM LY294002 significantly inhibited glioblastoma migration/invasion by ~27% within the glioblastoma cell mass (0-80 µm) and by ~32% compare to control at further distances (81-160 µm). These results are consistent with effects observed in orthotopic murine models of glioblastoma testing LY294002 and demonstrate that we have a short duration, relevant and sensitive embryo larval zebrafish assay to prioritize glioblastoma therapeutic development. Funding provided by NSF1134468 and NIH T32 ES07060 and P30 ES000210.

1805 Development of a Larval Zebrafish Neurobehavioral Assay for Evaluating Narcotic-Like Effects of Alcohols and Alcohol Ethoxylate Surfactants

S. M. Bugel[2], H. W. Broening[1], L. Truong[2], J. K. La Du[2], G. Carr[1], J. F. Nash[1], G. Daston[1] and R. L. Tanguay[2]. [1]*The Procter & Gamble Company, Cincinnati, OH and* [2]*Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR.* Sponsor: S. Bugel.

Several alcohol ethoxylate (AE) surfactants have been shown to induce narcosis in higher vertebrates. To rapidly screen and evaluate the narcotic-like effects of AE surfactants, a neurobehavioral assay was developed using zebrafish. The assay is based on locomotor responses stimulated by a light-to-dark phototransition in 5 day post fertilization (dpf) larvae. Narcosis is measured as a concentration-dependent decrease in locomotor activity. Zebrafish are exposed beginning at 5 dpf to each test material for 30 min prior to being subjected to 4 trials of alternating light/dark periods, each photoperiod lasting 3 minutes. The purpose of these studies was to (1) conduct a proof-of-principle experiment using a series of straight-chain alcohols (C6-C16) with known narcotic activity in a broad range of species and tissue preparations, and (2) investigate AE surfactants for narcotic activity. The C6-C12 alcohols induced narcosis and inhibited photolocomotor responses in the larval zebrafish with IC50s ranging from 14.4 to 370 µM. There was a clear structure-activity relationship between carbon number and narcotic potency, with the C10 alcohol exhibiting the greatest degree of narcotic potency in this alcohol series. However, narcosis was not observed with the C13-C16 alcohols, possibly due to limitations with the water solubility of these alcohols. An ethoxylated analog of the C12 alcohol (Thesit®, C12EO9) exhibited the greatest narcotic potency of the materials tested. The narcotic potency of the C12EO9 (IC50 = 4.7 µM) was approximately 13-fold greater than that of the parent C12 alcohol (IC50 = 62.6 µM). These studies collectively demonstrate the utility of the larval zebrafish model to detect and characterize narcotic agents, including AE surfactants. Supported by: P30 ES00210 and T32 ES07060.

1806 Development of Zebrafish Models for Human Acute Organophosphorus Poisoning

N. Garcia-Reyero[4], M. Faria[5], F. Padrós[10], P. J. Babin[8], D. Sebastián[3], J. Cachot[7], E. Prats[1], M. Arick[9], E. Rial[6], A. Knoll-Gellida[8], G. Mathieu[8], F. Le Bihanic[8], B. L. Escalon[4], A. Zorzano[3], A. M. Soares[5] and D. Raldúa[2]. *[1]CID-CSIC, Barcelona, Spain; [2]IDÆA-CSIC, Barcelona, Spain; [3]Institute for Research in Biomedicine, Barcelona, Spain; [4]US Army ERDC, Vicksburg, MS; [5]Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal; [6]Department of Cellular and Molecular Medicine, CIB-CSIC, Madrid, Spain; [7]EPOC, Université de Bordeaux, Barcelona, Spain; [8]EPOC, Université de Bordeaux, Talence, France; [9]Institute for Genomics, Biocomputing & Biotechnology, Mississippi State University, Starkville, MS and [10]Pathological Diagnostic Service in Fish, Universitat Autònoma de Barcelona, Bellaterra, Spain; [11]Rare Diseases, Genetic and Metabolism, Université de Bordeaux, Talence, France.* Sponsor: E. Perkins.

OOrganophosphorus compounds (OP) are commonly used pesticides with nearly 3 million cases of acute poisoning and 300,000 deaths world wide, annually. The molecular initiating event leading to poisoning is inhibition of acetylcholinesterase (AChE), resulting in the accumulation of the neurotransmitter acetylcholine (ACh) in the cholinergic synapses. However, the pathways leading to the adverse outcomes are not well-understood. Zebrafish is being increasingly used as a model species for both ecological and human health risk assessment. Previous studies on the modes of toxic action (MoA) for these pesticides have been analysed in zebrafish embryos but have been confounded by developmental impacts in addition to toxic effects. In this study we use a prototypic OP, chlorpyrifos-oxon (CPO), to develop a zebrafish model of OP poisoning using a set of molecular markers. The acute effects of mild, moderate, and high CPO concentrations on zebrafish larvae have been studied at different levels of organization including molecular (transcriptomic analysis by RNAseq, biochemical responses), cellular, tissue and organismal (gross morphology, behavioral effects [visual motor response (VMR) and touch-evoked escape response]). Three phenotypes were identified by gross-morphology and behavioral analysis. In conclusion, the adverse outcome pathways of the different phenotypes have been dissected empirically and the results obtained show that exposed zebrafish mimic many aspects of the human OP poisoning, confirming zebrafish larvae as a suitable model to identify new antidotes against cholinergic syndrome.

1807 Identification of Neuromast Disruptor Compounds Through *In Vivo* Screening in Zebrafish

R. Sachanandani[1], S. Thrikawala[1], J.-k. Gustafsson[1] and M. Bondesson[2]. *[1]Department of Biology and Biochemistry, University of Houston, Department of Biology and Biochemistry, Center for Nuclear Receptors and Cell Signaling, Houston, TX and [2]Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Department of Pharmacological and Pharmaceutical Sciences, Houston, TX.* Sponsor: D. Zalko.

Many industrial chemicals such as pesticides are released into the environment without a full assessment of their adverse effects on human life and the ecosystem. We aim to understand the developmental toxicity effects of environmental pollutants by using the vertebrate zebrafish (*Danio rerio*) as a model to identify neuromast disruptors. The zebrafish lateral line responds to the flow of water via receptors called neuromasts, which consist of a cluster of hair cells surrounded by a ring of supporting cells and innervated by sensory neurons. Neuromasts are positioned along the zebrafish body through migration of the neuromast primordium formed in the head region. The same family of chemokine receptors may be responsible for neuromast migration and metastasis of certain cancer cells (Galardo et al., Disease Models & Mechanisms (2015) 8, 565-576). Transgenic zebrafish embryos expressing fluorescent markers in neuromasts were used to visually monitor neuromast development under normal and perturbed conditions using the control compounds AG1478 and Valproic Acid (VPA). Next, these embryos were used in a primary screen of 305 compounds of the ToxCast phase I chemical inventory, primarily consisting of pesticides and antimicrobials. Chemicals that perturbed or delayed neuromast migration were re-screened in zebrafish embryos that had been injected with the breast cancer cell line MDA-MB-231 to investigate the effect of the chemicals on micrometastasis. In conclusion, our results show that several environmental pollutants have neuromast disrupting capacity and that the developing zebrafish embryo is an efficient *in vivo* model that can be used for identification of compounds interfering with cell migration.

1808 Optimizing Assays to Identify Adult Zebrafish Behavioral Phenotypes

A. J. Garcia, L. Truong, R. Tanguay and E. Johnson. *Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

The use of zebrafish in behavioral neuroscience is rapidly growing. Zebrafish can be assessed for alterations in multiple behavioral endpoints, creating opportunities to use this powerful model to identify chemicals that alter behavioral phenotypes. To evaluate the utility of zebrafish for neurotoxicity research, we designed custom instrumentation to evaluate numerous adult zebrafish behaviors. Shuttleboxes were used to evaluate learning and active avoidance conditioning and a zebrafish Visual Imaging System (zVIS) was used to measure social behavior and fear responses. As proof of concept, we used this instrumentation to measure the impact of Vitamin E deficiency on complex adult behaviors. Zebrafish were fed defined-diets that either had sufficient or deficient levels of Vitamin E. The vitamin E deficient zebrafish had a ~30% decrease in learning rate relative to the fish with sufficient levels of Vitamin E. The zVIS system consists of an array of 8 tanks with only single side views of video projections on LCD monitors. This allows individual fish to visualize either a group of swimming zebrafish or single predator fish. For the socialization assay, the expectation is that test zebrafish will normally socialize, or go nearer to the screen. For the predator test, zebrafish are expected to move away from the screen. The proximity of the zebrafish are tracked relative to the LCD screen projections. The preliminary results from non-exposed zebrafish showed the percent of time spent near the screen during the socialization assay was ~65%. For the predator test the fish the fish spend 75% of their time away from the screen. Collectively, these data demonstrates that custom behavioral systems are able to measure complex behavioral phenotypes and suggests that there are enormous opportunities for translation neurotoxicity research using zebrafish.

1809 Developmental Toxicity From Exposure to Various Forms of Mercury Compounds in the Medaka Fish (*Oryzias latipes*) Embryos

W. Dong[1,2], J. Liu[4], L. Wei[3], J. Yang[1] and D. E. Hinton[2]. *[1]Inner Mongolia Provincial Key Laboratory for Toxicants and Animal Disease/Inner Mongolia University for the Nationalities, Tongliao, China; [2]Nicholas School of the Environment, Duke University, Durham, NC; [3]Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China and [4]Zunyi Medical College, Zunyi, China.*

Mercury is a toxic heavy metal that can become incorporated in marine fish and be a dietary concern for women of reproductive age. This study examined the developmental toxicity of different mercury compounds in medaka fish embryos. Medaka embryos were exposed to 0.001-10 μM concentrations of MeHg, HgCl2, α-HgS (cinnabar), and β-HgS (Zuotai) from the Stage 10 to 10 days post fertilization (10 dpf). Of the forms of mercury, the methylated form (MeHg) is organic, readily crosses cell membranes and proved MeHg is highly toxic. Inorganic mercury (HgCl2) was less toxic but produced embryo death and developmental toxicity in our fish model. This includes pericardial edema, elongated or tube heart, malformation of eye and failure of inflation in swim bladder. In contrast, both α-HgS, and β-HgS were much less toxic than either of the above. Total RNA was extracted 3 days after exposure of embryos to MeHg (0.1 μM), HgCl2 (1 μM), α-HgS (10 μM), or β-HgS (10 μM) to examine toxicity-related gene expression. MeHg and HgCl2 markedly induced metallothionein and heme oxygenase-1, while α-HgS and β-HgS did not produce significant changes in either gene. In conclusion, chemical forms of mercury compounds are a major determinant of their developmental toxicity towards medaka embryos. The medaka embryo model proved capable of detecting effects rapidly and results differentiated between various forms of mercury.

1810 Abstract Withdrawn by Author.

1811 Assessment of *C. elegans In Vivo* ATP Status and Larval Development After Exposure to a Subset of Tox21 Compounds

J. R. Rice[3], P. E. Dunlap[3], M. V. Smith[1], M. F. Bridge[1], J. H. Freedman[2], J. Zhao[4], R. Huang[4], M. S. Attene-Ramos[4], M. Xia[4], A. Simeonov[4] and W. A. Boyd[3]. *[1]Social & Scientific Systems, Inc., Durham, NC; [2]University of Louisville, Louisville, KY; [3]DNTP, NIEHS, Durham, NC and [4]NCATS, NIH, Bethesda, MD; [5]NCATS, NIH, Bethesda, MN.*

The Tox21 program is a partnership of the National Toxicology Program (NTP), the US Environmental Protection Agency (EPA), the National Center for Advancing Translational Sciences (NCATS) and the Food and Drug Administration (FDA) focused on screening environmental chemicals for potential toxicity. Previously, the Tox21 10K library containing ~8300 unique chemicals was screened for mitochondrial membrane potential (MMP) and cell viability in HepG2 cells. Follow-up studies were performed to further characterize the Tox21 compounds found active in the MMP screen and to prioritize chemicals for study in traditional *in vivo* assays. In follow-up, 63 Tox21 compounds were tested in the nematode *Caenorhabditis elegans* using two assays: larval growth and development, which utilized wild type nematodes to measure size and developmental stage after 48 h exposures; and *in vivo* ATP status, which used a transgenic *C. elegans* strain that constitutively expresses firefly luciferase to provide real time measurements of ATP after 24 h exposures. Compounds were tested at 9 concentrations ranging from 0.5 µM to 100 µM with a few highly potent compounds tested at additional lower concentrations until no effect was observed. Similar numbers of compounds were found to be active in both assays with 45/63 and 41/63 chemicals affecting larval development and ATP status, respectively. However, the potencies varied such that some compounds were active at lower concentrations in the larval growth and development assay, and some in the ATP assay, while others had similar LECs in both assays. The most potent compound was pinacyanol chloride, a water-soluble, dichroic dye active at 5 nM; while the most active compound tested was the pesticide chlorfenapyr, which caused the greatest reduction in size and ATP status.

1812 The BPA Substitute Bisphenol S Induces Distinct Alterations of Germline Function

Y. Chen[3], D. Y. Lee[3], L. Shu[3], X. Yang[3] and P. Allard[3]. *[1]Department of Environmental Health Sciences, University of California, Los Angeles, CA; [2]Department of Integrative Biology and Physiology, University of California, Los Angeles, CA and [3]Institute for Society and Genetics, University of California, Los Angeles, CA.*

Concerns about the safety of Bisphenol A, a chemical found in plastics, receipts, food packaging and more, have led to its replacement with substitutes now found in a multitude of consumer products. However, several popular "BPA-free" alternatives, such as Bisphenol S, share a high degree of structural similarity with BPA, suggesting that these substitutes may disrupt similar developmental and reproductive pathways. We compared the effects of BPA and BPS on reproductive endpoints using the nematode *C. elegans*, a canonical model for the study of germline function and meiotic differentiation. We found that BPS causes a dramatic increase in germline apoptosis and embryonic death comparable to BPA's, despite a lower tissue uptake. However, the germine effects of BPS are mainly achieved via global transcriptional changes and germline responses that are distinct from those elicited by BPA, including alterations of DNA damage and recombination pathways. These findings, along with a strong effect on embryonic viability when BPA and BPS are used in combination, raise new concerns about the safety of BPA alternatives and about the risk associated with human exposure to mixtures of these chemicals.

1813 Developmental and Behavioral Effects Following Chronic Exposure to Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) in *Caenorhabditis elegans*

J. South and C. A. Dodd. *Biology, Fort Valley State University, Fort Valley, GA.*

Organophosphate flame retardants (OPFRs) were introduced as alternatives to the recently phased out polybrominated diphenyl ethers. However, metabolites of a common OPFR, Tris(1,3-dichloro-2-propyl) phosphate (TDCPP), have been detected in human urine, indicating that OPFRs bioaccumulate. Limited data exist on the potential of OPFRs to act as developmental toxicants. This study aims to determine the developmental consequences of chronic exposure of TDCPP using *Caenorhabditis elegans*. N2 (wild-type) *C. elegans* were grown on NGM plates seeded with antibiotic resistant *E. coli* (OP-50). Following synchronization, L1 stage worms were collected in buffer and treated with TDCPP (0, 150 nM, 300 nM, 600 nM, 1.25 µM, 2.5 µM, 5 µM, 10 µM, 20 µM, 50 µM, 100 µM, and 1000 µM). Treated worms were plated for assessment of survival, head-tail development, and changes in behavior. Treatment with TDCPP (1.25 µM - 1000 µM) significantly decreased the percentage of live worms (20-40%) compared to control. Motor activity was significantly increased at concentrations as low as 5 µM, while head to tail development was altered at concentrations as low as 150 nM. Additionally, the vulva region of the hermaphroditic nematodes was underdeveloped at concentrations higher than 10 uM. Data from this study suggests that OPFRs have significant developmental effects on *C. elegans*. Due to the homologies of many genes important to development between *C. elegans* and humans it is feasible to predict that TDCPP may have developmental consequences at low environmental concentrations. Future studies will attempt to identify the mechanism of such developmental abnormalities induced by TDCPP.

1814 Transgenerational Effects of Aflatoxin B1 on the Nematode *Caenorhabditis elegans*

K. T. Sunmola, W. Feng, L. Tang and J.-S. Wang. *University of Georgia, Athens, GA.*

Aflatoxin B1 (AFB1), a naturally occurring mycotoxin produced by toxigenic fungi *Aspergillus flavus* and *A. parasiticus*, is one of the most common food and feed contaminants in the developing world. AFB1 is a potent hepatotoxic and genotoxic agent, and is identified as a class 1 human carcinogen. The toxic effects of AFB1 have been studied extensively in various animal models. However, most of toxic effects were observed within one generation of the test organisms. The aim of this study was to examine the possible transgenerational effects of AFB1 *in vivo* using the nematode *Caenorhabditis elegans*. The wildtype (N2 Bristol) parent worms (F0 generation) were treated with 0, 10, 30, and 90 µmol/L AFB1. The effects of AFB1 treatment on growth, generation time, and brood size in F0 generation, their first generation offspring (F1) and second generation offspring (F2) of the nematodes were explored. After AFB1 exposure, reproduction in the F0 generation was impaired as indicated by the significantly reduced brood size and extended generation time. The brood size reduced by 46.5%, 89% and 91.6% in the 10, 30 and 90 µmol/L AFB1-treated groups respectively compared to the control group. Mean generation time showed a significant 2-fold increase ($p<0.01$) in the group treated with 90 µmol/L AFB1. Also, development of the nematodes was affected by AFB1 exposure, as indicated by the significantly reduced body length, with 33%, 40% and 53% decreases in the 10, 30 and 90 µmol/L AFB1-treated groups respectively compared to the control. Significant toxic effects found in AFB1-treated parent F0 generation were also observed in their F1 offspring. The brood size in F1 offspring decreased by 48% and 91% in the nematodes treated with 30 and 90 µmol/L AFB1. Decreases in body lengths were also found in F1 offspring whose parents (F0) were treated with AFB1, but not dose-dependent. F2 offspring appears unaffected in reproduction and growth whether their F0 generation was AFB1-exposed or not. Data from this study suggests the likelihood that the AFB1-induced reproductive and developmental toxic effects in F0 nematodes may, in some extent, transfer to their immediate F1 offspring, but such toxic effects may not extend to the F2 offspring.

1815 MicroRNAs As Biomarker of Toxicity Effects of Chemical Dispersed Oil Pollution in *Caenorhabditis elegans*

Y. Zhang and X. Pan. *Biology, East Carolina University, Greenville, NC.*

Backgroud: The BP oil spill is among the most severe environmental disasters in US history. The nematode *Caenorhabditis elegans* (*C. elegans*) has been a useful tool for environmental toxicity studies. In previous study, we found the crude oil and/or dispersant induce growth inhibition and offspring reduction in *C. elegans*. At the molecular level, microRNA-mediated gene silencing has emerged as a fundamental regulatory mechanism of gene expression in many biological processes. However, miRNAs-mediated gene regulation in response to major pollution events is poorly understood. Methods: Here we systematically investigated a total of 231 microRNA expression by using qRT-PCR and analyzed the expression profile of miRNAs in *C. elegans* in response to oil-alone, dispersant-alone and the mixture of oil and dispersant. TargetScan6.2 and Miranda were applied to predict the targets of differentially expressed miRNA genes in each treatment individually. Then, a further target function analysis was performed based on the KEGG pathway database. Results: The aberrant expressions of miRNAs were induced and KEGG pathway enrichment analyses indicated that those significantly changed miRNA expression affect many biological processes in *C. elegans*. Many affected pathways are related to environmen-

tal information processing, such as ABC transporters, MAPK signaling pathway, Erbb signaling pathway, JAK-STAT signaling pathway, MTOR signaling pathway and calcium-signaling pathway. Some pathways are related to oil uptake and metabolism, such as endocytosis, fatty acid biosynthesis and phosphatidylinositol signaling system. Conclusion: Based on the genome-wide investigation of microRNA profile, it suggests that *C. elegans* may respond to environmental stimuli, like oil, dispersant and oil-dis mixture and activate many pathways. These pathways are related to many important biological processes, such as cell cycle, cell proliferation, differentiation and apoptosis. Since the currently identified proteins and microRNAs in *C. elegans* show remarkable conservation with mammals including humans, the oil/dispersant may also induce similar change in microRNAs expression and affect many biological processes that lead to reproduction toxicity.

PS 1816 Influence of Maternal Age on the Effects of Organic Selenium in *Daphnia pulex*

J. E. Roberts[2], T. S. Schwartz[1] and J. M. Gohlke[2,3]. [1]*Department of Biological Sciences, Auburn University, Auburn, AL;* [2]*Department of Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL and* [3]*Department of Population Health Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.*

As a necessary micronutrient, seleno-L-methionine exhibits a U-shaped dose response with deficiency and toxicity leading to adverse health outcomes. Some studies suggest dietary selenium may offer protection against developmental toxicity from other metals, but excessive dietary levels can also lead to toxicity. In addition, increased maternal age has been shown to increase the risk of adverse developmental outcomes. We hypothesize that older maternal age will increase the susceptibility of offspring exposed to toxic doses of seleno-L-methionine. Therefore, the goal of this project was to evaluate the effects of seleno-L-methionine dose on lifespan and determine if the effects are altered by maternal age using *Daphnia pulex* as the model system. Offspring were obtained from our maternal populations (N=4) when the mothers were 8 days of age (early life brood) and when the mothers were 32 days of age (late life brood). The offspring from each brood were randomized into the 0 µg Se/L, 1.02 µg Se/L, and 4.08 µg Se/L dose groups. The effect of selenium dose on the offspring from the early and late life broods was quantified using lifespan measurements (N=152). Selenium tissue content (N=36) was measured in 15 day old offspring to verify selenium absorption. The 4.08 µg Se/L dose groups had significantly higher selenium tissue content than the 0 µg Se/L dose groups ($p=0.0061$) and the 1.02 µg Se/L dose groups ($p=0.0203$), however, there was not a statistically significant difference between the 0 µg Se/L dose groups and the 1.02 µg Se/L dose groups. The offspring from the highest dose groups (4.08 µg Se/L) did not reproduce and had significantly shorter lifespans ($p<0.0001$) in comparison to the 0 µg Se/L and 1.02 µg Se/L dose groups. However, there was not a significant effect of maternal age. Time to first reproduction was delayed in the intermediate dose group (1.02 µg Se/L, $p<0.0001$). Within the 1.02 µg Se/L dose groups, the offspring from the later life brood began reproducing later than the offspring from the early life brood ($p=0.0063$). These results suggest that, at the age range tested, there is a detectable influence of maternal age on the effect of seleno-L-methionine for the onset of reproduction, but there is not a detectable influence on lifespan in *Daphnia pulex*.

PS 1817 Luteolin Protects Against Methylmercury-induced Biochemical and Neurobehavioral Deficits in Lobster Cockroach *Nauphoeta cinerea*

I. A. Adedara[1,2], D. B. Rosemberg[1], D. O. Souza[4], E. O. Farombi[2], M. Aschner[5] and J. B. Rocha[1]. [1]*Universidade Federal de Santa Maria, RS, Brazil, Santa Maria, Brazil;* [2]*Biochemistry, University of Ibadan, Ibadan, Nigeria;* [3]*Departamento de Bioquímica e Biologia Molecular, CCNE, Universidade Federal de Santa Maria, RS, Brazil, Santa Maria, Brazil;* [4]*Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, RS, Brazil, Santa Maria, Brazil and* [5]*Department of Molecular Pharmacology, Albert Einstein College of Medicine, New York, NY.*

Luteolin (3', 4', 5, 7-tetrahydroxyflavone) is a polyphenolic compound found in foods of plant origin and has been reported to possess antioxidant, anti-inflammatory and neuroprotective properties. However, there is dearth of information on the beneficial effects of luteolin on methylmercury (MeHg)-induced neurotoxicity. This study evaluated the influence of luteolin on MeHg-induced behavioral and biochemical deficits using lobster cockroach *Nauphoeta cinerea* as an alternative to utilizing conventional animal models. The insects were exposed to either MeHg alone (0.05 mg/g feed) or in combination with luteolin at 0.25, 0.5 and 1.0 mg/g feed for 35 days. Locomotor behavior was assessed using video-tracking software during a 10-minute trial in a novel arena and subsequently, biochemical analyses were carried out using the cockroaches heads. Luteolin dose-dependently reversed MeHg mediated locomotor deficits evidenced by the improvement in the total distance travelled, time spent walking, maximum speed, body rotation and turn angle whereas it decreased the number of falls, time and episodes of immobility. The enhancement of the exploratory profiles of MeHg-exposed cockroaches by luteolin was confirmed by track and occupancy plots analyses. Moreover, luteolin prevented MeHg-induced acetylcholinesterase activity inhibition, decreased dichlorofluorescein oxidation and lipid peroxidation levels but increased the antioxidant status in the treated cockroaches. In conclusion, luteolin prevented oxidative stress indices and neurobehavioral deficits in *Nauphoeta cinerea* model of MeHg toxicity.

PS 1818 Comparison of Novel *In Vitro* Systems for the Detection of Ciguatoxins and Their Use in Risk Extrapolation to Humans

I. Richter[1,2], A. H. Heussner[2] and D. R. Dietrich[2]. [1]*Cawthron Institute, Nelson, New Zealand and* [2]*Human and Environmental Toxicology, University of Konstanz, Konstanz, Germany.*

Ciguatera fish poisoning in humans is the most common marine biotoxin food poisoning worldwide with up to 500,000 people affected annually. Intoxication is caused by ciguatoxins (CTXs), which occur in fish as a result of biotransformation of the microalgal precursor gambiertoxin. Detection and quantification of CTXs remains difficult due to the simultaneous presence of multiple toxin congeners and their metabolites. Although several *in vitro* methods exist (e.g. murine neuroblastoma N2a cell line), the most widely used assay for the detection of CTX toxins in fish is the *in vivo* mouse bioassay (MBA). For the detection of marine biotoxins in seafood in the EU from 2015 onwards, the MBA will be banned and replaced by LC-MS analysis. Thus there is an urgent need for *in vitro* methods that assess biological endpoints to contrast the analytical methods. Recently, a novel *in vitro* yeast bioassay, based on tunicate xenobiotic receptors, was described which is sensitive to marine biotoxins (e.g. okadaic acid, pectenotoxin-11)[1]. This study aimed to develop this novel *in vitro* yeast bioassay into an efficient, broad-spectrum screening tool for the detection of CTX congeners and their metabolites. For this, a set of different neurotoxic compounds (e.g. n-butyl-p-aminobenzoate, carbamazepine, brevetoxins and CTXs) was tested in the yeast bioassay and the commonly used N2a assay (48 hour MTT reduction assay). The two positive controls (n-butyl-p-aminobenzoate, carbamazepine) were active in both *in vitro* assays (n-butyl-p-aminobenzoate: yeast EC50 = 124±1 µM; N2a EC50 = 48±2 µM; carbamazepine: yeast EC50 = 6±1 µM; N2a EC50 = 365±1 µM), albeit with different sensitivity, suggesting that the tunicate yeast bioassay could serve as an inexpensive and rapid tool for the detection of neurotoxic compounds. 1.Richter, I., Fidler, A. Toxicon, 2015. 95(0).

PS 1819 Inhibition of Endocannabinoid Degradation Interferes with Cephalic Regeneration in *Girardia tigrina*

R. Sneed. *Division of Science and Mathematics, University of the District of Columbia, Washington, DC.*

The endocannabinoid system is a major regulator of physiologic functions in mammals such as pain, memory, and feeding behavior. In previous studies in our laboratory, treatment with the synthetic cannabinoid WIN 55,212-2 inhibited cephalic regeneration in the North American brown planarian *Girardia tigrina* (Girard, 1850). To further define the role of endocannabinoids in planarians, we focused on the enzymes regulating the breakdown of the endogenous cannabinoids, N-arachidonoylethanoamine (anandamide; AEA) and 2-arachidonoylglycerol, which serves as the major mechanism for termination of endocannabinoid action. Fatty acid amide hydrolase (FAAH) is specific for N-arachidonoylethanoamine and related N-acylethanolamine-type compounds (i.e., N-palmaloylethanolamine and N-stearoylethanolamine) while 2-arachidonoylglycerol is degraded by monoacylglycerol lipase (MAGL). We hypothesize that inhibition of endocannabinoid degradation will produce an effect similar to treatment with exogenous cannabinoids due both to prolongation of action and increased levels of endogenous cannabinoids. Decapitated planarians (n=20 per group) were treated with either normal media, DMSO solvent control, PF-3845 (up to 4.5 micromolar), URB597 (up to 20 micromolar) (both inhibitors of FAAH), or KML29 (up to 40 micromolar) (an inhibitor of MAGL) over a period of 5 days, the normal period of cephalic regeneration. To measure the effects on cephalic regeneration, planarians were stained with 1 percent acetocarmine and images were recorded using a digital compound light microscope. Planarians treated with either PF-3845 or

URB597 had significantly reduced cephalic regeneration compared to controls; however, cephalic regeneration in KML29-treated animals was not different from controls. These data suggest that the primary endocannabionoid found in planarians is N-arachidonoylethanoamine or a closely related compound. Future studies will seek to measure the levels of N-arachidonoylethanoamine and the other ethanolamines thought to be significant in the endocannabinoid system. [Supported by NIH Award T36GM101997-02].

1820 Genotoxic Potential of Amino- and Nitro-Substituted Mono- and Polycyclic Aromatic Amines in Chicken Egg Genotoxicity Assay (CEGA)

G. M. Williams[2], T. Kobets[2], J. D. Duan[2], K. D. Brunnemann[2], M. J. Iatropoulos[2], E. Vock[1] and U. Deschl[1]. *[1]Boehringer Ingelheim Pharma GmbH & Co, Biberach an der Riss, Germany and [2]Pathology, New York Medical College, Valhala, NY.*

Amino- and nitro-substituted mono- and polycyclic aromatic amines were tested in Chicken Egg Genotoxicity Assay (CEGA) using comet assay to detect DNA strand breaks and nucleotide 32P-postlabelling (NPL) assay to detect DNA adducts in fetal chicken livers. Fertilized white chicken eggs received 3 daily injections with test compounds on days 9-11 of incubation and tests were conducted 3 hours after the last dosing. Among monocyclic compounds, aniline produced no DNA strand breaks or adducts. In contrast, the methyl-substituted carcinogenic anilines, 2,6-xylidine and o-toluidine, were positive in both assays. The local anesthetics lidocaine and prilocaine, which are metabolized to 2,6-xylidine and o-toluidine, respectively, were positive in at least one of the assays. The analgesics acetaminophen and phenacetin did not produce DNA damage. The carcinogen para-cresidine induced a weak response in comet assay. Among the polycyclic compounds, fluorene produced no DNA damage. The substituted compounds, 1-aminofluorene, 2-aminofluorene, 2-nitrofluorene and the carcinogen 2-acetylaminofluorene, were positive in both assays. Naphthalene, which has no amino-substitution, was negative in CEGA. 1-Naphthylamine was also negative, whereas its isomer, the carcinogen 2-naphthylamine was positive. Unsubstituted biphenyl was negative in both assays, while carcinogen 4-aminobiphenyl was positive. Thus, our results showed that compounds positive in NPL were also positive in comet with the exception of prilocaine. Also, NPL data show that polycyclic amines were more potent compared to monocyclic compounds. Furthermore, methyl-, amino-, acetylamino- and nitro- substitutions had higher genotoxic potential compared to prototype aromatic compounds. While there is a possibility that the results for several compounds could have been affected by their low solubility, the majority of the outcomes were consistent with previous studies, providing more evidence for reliability of the model.

1821 Structure-Activity Relationship in Genotoxicity of Alkenylbenzenes in Turkey and Chicken Egg Fetal Liver

T. Kobets[3], J. D. Duan[3], K. D. Brunnemann[3], S. Etter[2], B. Smith[2], E. Vock[1], U. Deschl[1] and G. M. Williams[3]. *[1]Boehringer Ingelheim Pharma GmbH & Co, Biberach an der Riss, Germany; [2]Firmenich Inc., Plainsboro, NJ and [3]Pathology, New York Medical College, Valhala, NY.*

Certain alkenylbenzenes (AB), flavoring chemicals naturally occurring in spices and herbs, are established to be cytotoxic and hepatocarcinogenic in rodents. The present study determined and compared the genotoxic potential of key representatives of this class using Turkey and Chicken Egg Genotoxicity Assays (TEGA and CEGA, respectively). In TEGA, medium white turkey eggs with 22-24 day old fetuses received 3 daily injections with nine AB of different carcinogenic potentials: safrole (1, 2 mg/egg), methyl eugenol (2, 4 mg/egg), estragole (20, 40 mg/egg), myristicin (25, 50 mg/egg), elemicin (20, 50 mg/egg), anethole (5, 10 mg/egg), methyl isoeugenol (40, 80 mg/egg), eugenol (1, 2.5 mg/egg) and isoeugenol (1, 4 mg/egg). In CEGA, white leghorn chicken eggs with 9-11 days old fetuses received 3 daily injections with safrole (4, 6 mg/egg), methyl eugenol (1, 2 mg/egg), and eugenol (1.5, 3 mg/egg). Three hours after the last injection, fetal livers were harvested for assessment of DNA strand breaks, using comet assay and DNA adducts formation, using nucleotide ^{32}P-postlabeling assay. In TEGA, estragole, myristicin and elemicin induced DNA strand breaks. These compounds as well as safrole, methyl eugenol and anethole, at the highest doses tested, formed DNA adducts. Methyl isoeugenol, eugenol and isoeugenol did not induce genotoxicity. Similarly, in CEGA, safrole and methyl eugenol induced DNA damage, while eugenol did not. The pattern of adducts in both models was specific for each compound. The genotoxic AB had the structural features of either a double bond in the alkenyl side chain at the terminal 2′,3′-position favorable to formation of proximate carcinogenic 1′-hydroxymetabolite or terminal epoxide, or absence on the benzene ring of a free phenolic hydroxyl group crucial for formation of a non-toxic glucuronide conjugate. In contrast, methyl isoeugenol, eugenol and isoeugenol, which were non-genotoxic, possessed chemical features, unfavorable to activation.

1822 Role of ATP Binding Cassette Sub-Family Member 2 (ABCG2) in Mouse Embryonic Stem Cell Development

M. Rosen[1], K. Chandler[2], S. Jeffay[1], S. Charla[1], H. Nichols[1], M. Hoopes[1] and E. S. Hunter[1]. *[1]Integrated Systems Biology, US EPA, Research Triangle Park, NC and [2]Office of Policy, Planning and Evaluation, NIEHS, Research Triangle Park, NC.*

ATP binding cassette sub-family member 2 (ABCG2), is a member of the ABC transporter superfamily and a principal xenobiotic transporter. ABCG2 is also highly expressed in certain stem cell populations where it is thought to be related to stem cell plasticity, although the role of ABCG2 during early embryonic development is not established. While ABCG2-null mice exhibit a normal phenotype, inhibition of ABCG2 in mouse embryonic stem cells (mESC) has been shown to reduce colony expansion and promote differentiation. Furthermore, our earlier evaluation of ToxCast Phase 1 chemicals suggested an association between xenobiotics that regulate Abcg2 transcription and altered differentiation of mESC. J1 cells were seeded onto 96 well plates and allowed to attach for 24 hrs. Cells were cultured for various days in combination with K0143, a specific ABCG2 inhibitor, and either chemicals predicted by ToxCast Phase I assays to regulate the transporter expression or known substrates of ABCG2 (mitoxantrone or topotecan). Cell number was determined by RedDot1 staining and ABCG2 transport activity assessed using pheophorbide a. Differentiation was evaluated by real-time qRT-PCR using Pou5f1, Nanog, T, Gsc, Bmp4, Nes, Ncam1, Des, Ttr, Gata4, Myl4, and Myl. K0143 treatment alone had no effect on mESC differentiation or cell number and did not alter the toxicity of chemicals shown to regulate Abcg2 transcription in ToxCast Phase I assays. K0143, on the other hand, increased the toxicity of mitoxantrone and topotecan. Hence, the hypotheses that ABCG2 plays a fundamental role in mESC differentiation or that regulation of the receptor may be a toxic mode of action could not be supported. ABCG2, however, does serve a protective role as a xenobiotic transporter during early mESC differentiation. This analysis aids in developing a predictive model for identification of developmental toxicants. (This abstract does not reflect US EPA policy.)

1823 Early Activation of Aryl Hydrocarbon Receptor Downregulates the Master Cardiac Transcription Factor Nkx2-5 and Upregulates Pacemaker Shox2

Q. Wang, C.-I. Ko, Y. Fan and A. Puga. *EH, University of Cincinnati, Cincinnati, OH.*

The aryl hydrocarbon receptor (AHR) is a critical mediator of gene-environment interactions. The prototypical ligand for the receptor is the environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Developmental exposure to TCDD significantly inhibited the formation of contractile cardiomyocyte nodes during embryonic stem (ES) cell differentiation. Genome-wide gene expression profiling suggests that activation of AHR by TCDD upregulates pacemaker Shox2 and downregulates master cardiac transcription factor Nkx2-5. It has been long noticed that Shox2 represses the expression of Nkx2-5 in the pacemaker region of the heart. To characterize molecular interactions between Ahr, Nkx2-5 and Shox2, we studied the consequences of TCDD exposure on the expression of Nkx2-5 and Shox2 during the differentiation of ES cells in culture. Nkx2-5 mRNA expression was detected in 3-day-old embryoid bodies (EBs) and gradually increased to a maximum by 9 days of differentiation. Shox2 expression was detected in 4-day-old EBs and maintained a constant level for the next 8-9 days. Using chromatin immunoprecipitation with antibodies to NKX2-5 and SHOX2, we found that activation of AHR by TCDD significantly inhibit the binding of the transcription factor to their downstream targets. Promoter driven reporter assay showed that TCDD exposure downregulated the promoter activity of Nkx2-5 and upregulated Shox2 promoter activity. Knockdown of Nkx2-5 or Shox2 by shRNA resulted in less sensitivity of the cells to TCDD toxicity. These data suggest that AHR activation by TCDD induce the repression of the key cardiac transcription factor Nkx2-5 and the upregulation of pacemaker Shox2. One important function of the AHR during development appears to be the coordination of a complex regulatory network responsible for attainment and maintenance of cardiovascular homeostasis. Supported by NIEHS 5R01 ES006273.

1824 Development of a New Cell-Based Assay Using Human ES/iPS Cell-Derived Neural Stem Cells for Developmental Neurotoxicity Testing

M. Suga[2], H. Kii[1], T. Uozumi[1], Y. Kiyota[1], H. Yamada[2] and M. K. Furue[2]. [1]*Nikon Corporation, Tokyo, Japan and* [2]*Laboratory of Stem Cell Cultures, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan;* [3]*Laboratory of Toxicogenomics Informatics, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan.* Sponsor: A. Hirose.

Human pluripotent stem cells, including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, could be a promising new tool for *in vitro* developmental neurotoxicity (DNT) testing. Recently, several DNT testing methods based on human ES cells have been proposed with "3R" concept; refining, reducing and replacing the DNT tests on vertebrata. However, animal-derived components such as Knockout Serum Replacement (KSR), B27 supplement, or Matrigel, are still used to derivate neuronal cells from human ES cells in the methods. Although the comprehensive transcriptomics analysis could dynamically detect response of gene expression to chemical exposure, more cost-effective and rapid screening method would be expected. To address these issues, we are working to develop a new ES/iPS cell based assay for DNT testing. Here we present a new method to induce neural stem cells from human ES/iPS cells under completely chemically defined culture condition without any animal-derived components. The neural stem cells derived by our new method were validated by their cell morphology and gene expression profile. We used an antiepileptic drug, Valproic acid (VPA), which is known to cause neural tube defects, to verify whether the ES/iPS cell derived neural stem cells could be suitable for usage on DNT testing. As a result, Valproate toxicity was detected at lower dose compared with that shown in previous reports. Further, calculating toxicity reduction by components in blood serum such as albumin might predict toxic potencies in humans from results on our cell-based-assay, because our culture condition consists of minimum essential components. Because of such high sensitivity of toxic detection, our cell-based-assay could be useful for rapid DNT testing.

1825 Humoral Factor From Rodent Astrocytes Promoted the Synaptic Transmission and Augmented Synchronized Population Bursts in Human Induced Pluripotent Stem Cell-Derived Neurons

N. Miyamoto and K. Sawada. *Biopharmaceutical Assessments Core Function Unit, Eisai Co., LTD, Tsukuba, Japan.* Sponsor: S. Asakura.

Many drugs have been reported to have the risk of seizures. The causes of drug-induced seizures are GABAA antagonism, GABAB agonism, adenosine antagonism, and enhanced excitation through NMDA, and so on, in the neurons. So far, there is no good *in vitro* assay system for predicting drug-induced unexpected seizure-risks. Spontaneous neuron activity recordings by multi-electrode array (MEA) system from networks of cultured neurons could be a good risk evaluation system for such drug-induced seizure events. It was reported that long-term electrophysiological activity and pharmacological response of human induced pluripotent stem cell (hiPSC)-derived neurons were accelerated by co-culture with rat astrocytes. In this study, we observed time course generation of population burst spikes from hiPSC-derived neurons with conditioned medium of mouse primary astrocytes by MEA system. Humoral factor(s) from mouse primary astrocytes was sufficient to generate synchronized population burst spikes in the multiple types of iPSC-derived neurons. GABA antagonism by GABAzine or picrotoxin enhanced those periodic synchronized burst spikes in a dose-dependent manner as well as primary rat cortex neurons. P/Q-type and N-type calcium channel blockers eliminated the periodic synchronized burst spikes, suggesting that the burst spikes are mediated by synaptic transmission. We concluded that the observed astrocyte-induced population bursts by MEA system are mediated by synaptic transmission and the periodic synchronized population burst signals could be a good prediction marker of GABAA antagonism by drugs. This research is partially supported by the grants for iPS Non-clinical Experiments for Nervous System (iNCENS) project in Research Grants on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and development, AMED.

1826 Pharmacological Responses in Cultured Human iPSC Derived Cortical Neurons Using Multi-Electrode Array

A. Odawara[3,4], H. Katoh[4], N. Matsuda[4], Y. Shi[2], H. Jiko[1] and I. Suzuki[4]. [1]*Alpha Med Scientific Co., Ltd, Osaka, Japan;* [2]*Axol Bioscience Co., Ltd., Cambridge, United Kingdom;* [3]*Japan Society for the Promotion of Science Research Fellow, Tokyo, Japan and* [4]*Department of Electronics, Tohoku Institute of Technology, Sendai, Miyagi, Japan.* Sponsor: P. McNutt.

Human induced pluripotent stem cell (hiPSC)-derived neurons may be effectively used for drug discovery and cell-based therapy. However, the immaturity of cultured human iPSC-derived neurons and the lack of established functional evaluation methods are problematic. We here used a multi-electrode array (MEA) system to investigate the effects of the co-culture of astrocytes with hiPSC-derived cortical neurons on the long-term culture, spontaneous firing activity, and drug responsiveness effects. The co-culture facilitated the long-term culture of hiPSC-derived neurons over 400 days and long-term spontaneous firing activity was also observed. After >3 months of culture, we observed synchronous burst firing activity due to synapse transmission within neuronal networks. Compared with rat neurons, hiPSC-derived neurons required longer time to mature functionally. In drug responsiveness, addition of the synapse antagonist bicuculline, CNQX, AP5 and agonist a kainic acid, L-gulutamate induced the significant changes of firing rate and synchronized burst firing patterns. Furthermore, administration of pentylentetrazole (PTZ) induced epilepsy phenomenon. Anti-epilepsy drug phenytoin and sodium valproate reduced epilepsy phenomenon. These results suggested that long-term electrophysiological measurements in hiPSC-derived neurons using a MEA system may be beneficial for clarifying the functions of human neuronal circuits and for drug screening applications.

1827 Toxicity Assessment by Mitochondrial Dynamics in Human iPS Cells

Y. Kanda, S. Yamada and Y. Sekino. *Division of Pharmacology, National Institute of Health Sciences, Setagaya-ku, Japan.*

Organotin compounds, such as tributyltin (TBT), are well-known endocrine disruptors (EDCs). After TBT coatings were banned, butyltin compounds including TBT have been reported to be detected at concentrations between 50 and 400nM in human blood. TBT is known to cause various forms of cytotoxicity, including developmental toxicity and immunotoxicity. However, the molecular mechanism of TBT toxicities at nM levels has not been fully understood. We have previously reported that TBT at nM levels inhibited cell proliferation and ATP content in human multipotent cell line NT2/D1 (1-3). In the present study, we examined the effect of TBT on mitochondrial function in human iPS cells. Similar to NT2/D1 cells, exposure with 50nM TBT inhibited ATP content in 253G1 iPS cell line. In contrast, tin acetate (TA), as a negative control, had little effects. We next focused on mitochondrial dynamics in iPS cells. Mitochondria is well known to change its morphology in response to environmental conditions and is regulated by fusion proteins (Mfn, Opa1) and fission proteins (Fis1, Drp1). We found that TBT at 50 nM induced mitochondrial fragmentation, stained by MitoTracker, in iPS cells. TA had little effects. In addition, TBT exposure for 72 h reduced the Mfn fusion proteins without changes in gene expression. The other mitochondrial dynamics proteins were not affected by TBT. These data suggest that TBT at nM levels impaired mitochondrial quality in human iPS cells. We are now examining the effects of other EDCs in iPS cells. Thus, mitochondrial dysfunction in human iPS cells might be useful endpoints for evaluation of chemicals including EDCs. References (1) Yamada et al., Metallomics. 5: 484-491, 2013. (2) Yamada et al., Scientific Reports 4: 5952, 2014. (3) Yamada et al., Metallomics. 7: 1240-1246, 2015.

1828 Assessing the Impact of Drug Treatment on Cardiomyocyte Function Through Combined Analysis of Beating, Metabolic Flux and Cellular Oxygenation

C. Carey[1], C. Bertinetti-Lapatki[2], A. Roth[2] and J. Hynes[1]. [1]*Luxcel Biosciences, Cork, Ireland and* [2]*Pharmaceutical Sciences, Pharma Research & Early Development, Roche Innovation Center, Basel, Switzerland.*

Cardiotoxicity is a significant cause of compound attrition in preclinical development, prompting the use of stem cell derived cardiomyocytes as an *in vitro* model for toxicity screening. Also required however, are informative multi-parametric high-throughput methods assessing relevant functional parameters, with mitochondrial dysfunction of partic-

ular importance due to its involvement in the etiology of drug-induced cardiotoxicity. Here we examine the feasibility of combining microelectrode-based contractility measurements with a microplate-based bioenergetics assessment to better characterize cellular responses to drug treatment. Beating is assessed on 96 well E-plates using the xCELLigence Cardio system (ACEA) while cell metabolism is measured on the same plate using multiplexed fluorometric measurements of O2 consumption (MitoXpress®-Xtra), glycolytic flux (pH-Xtra) and cellular oxygenation (MitoXpress®-Intra); important parameters due to the dependence of cardiac tissue on oxidative metabolism and the involvement of O2 tension in cardiac gene regulation. Proof of concept data is presented using a panel of classical electron transport chain (ETC) modulators including antimycin, rotenone and FCCP, demonstrating that time-resolved fluorescence measurements can be performed on E-plates. Antimycin and rotenone impair beating and immediately inhibit ETC activity while cell-specific calibrations reveal increased cell oxygenation. In contrast FCCP causes increased ETC activity resulting in significantly decreased oxygenation. Cells were then treated with a panel of relevant compounds including the β-adrenoreceptor agonist isoproterenol, hERG channel inhibitors E-4031 and amiodarone and the L-type Ca2+ channel antagonist nifedipine, with impacts on beating, cell metabolism and oxygenation determined. This combined analysis of critical cardiomyocyte functions delivers increased mechanistic information and provides a more holistic cardiotoxicity screen.

PS 1829 Impedance and Combined Extracellular Field Potential Recordings of CIPA Reference Compounds on iPS Cardiomyocytes

C. T. Bot[2], S. Stölzle-Feix[1], N. Becker[1], U. Thomas[1], K. Juhasz[1], L. Doerr[1], M. Rapedius[1], M. Beckler[1], M. George[1], A. Brüggemann[1], R. Haedo[2], J. Oestreich[2], G. Okeyo[2], J. Costantin[2] and N. Fertig[1]. *[1]Nanion Technologies GMBH, Munich, Germany and [2]Nanion Technologies Inc, Livingston, NJ.*

Drug induced arrhythmia is one of the most common causes of drug development failure. Human induced pluripotent stem cell-derived cardiomyocytes (iPSCMs) show great promise for cardiovascular research and predictive *in vitro* cardiac safety screening. We present data recorded on the CardioExcyte 96, a hybrid screening instrument that combines impedance (cell contractility) with MEA-like extracellular field potential (EFP) recordings. A case study showing impaired excitation-contraction coupling will be presented. This indicates the importance of testing compounds in assays complementary to patch clamp electrophysiology to provide a more complete safety profile. Furthermore, in accordance with the Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) guidelines, standard reference compounds were tested on iPS-derived cardiomyocytes. Changes in the impedance signal indicate effects on cell contractility and shape whereas the field potential parameters provide information about the electrophysiological activity of the beating network of cells. Pharmacological effects of a number of reference compounds including those designated high risk(e.g. Dofetilide), intermediate risk (e.g. Cisapride) or low risk (e.g. Verapamil) were evaluated and summarized in this study. The CardioExcyte 96 system provides a non-invasive, label-free, high temporal resolution approach for safety screening. It can be either used in an incubator or, by utilizing the dedicated incubation system, directly on a lab bench. Furthermore, the system is capable of electrically pacing the cells, which allows for screening of cells which beat with individual frequencies and, in addition, investigations of frequency dependent compound inhibition.

PS 1830 Improved Functional Maturation of Human Induced Pluripotent Stem Cell (hiPSC) Derived Cardiomyocytes *In Vitro*: Multi-Electrode Array (MEA) Based Electrophysiological Characterization

M. L. Vlaming[1], F. Stevenhagen[1], T. de Korte[1], S. C. den Hartogh[1], K. Langenberg[1], M. Dunska[1], L. G. Tertoolen[2], C. L. Mummery[2], C. Grandela[1] and S. R. Braam[1]. *[1]Pluriomics B.V., Leiden, Netherlands and [2]Anatomy and Embryology, Leiden University Medical Centre, Leiden, Netherlands.* Sponsor: J. Louisse.

In drug development it is important to assess potential cardiotoxicity of drug candidates. hiPSC-derived cardiomyocytes are increasingly used for this purpose, for example using MEA- and/or impedance-based measurements to investigate drug effects on (electro)physiology. We have recently developed a serum-free maturation medium (Pluricyte® Cardiomyocyte Medium, PCM) and hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) which exhibit a relatively high level of maturity. This was demonstrated by an increased contraction profile, as well as electrophysiological properties (negative membrane resting potential, well defined action potential plateau and rapid depolarization) and gene expression patterns comparable to mature cardiomyocytes [1]. To further investigate the (electro)physiology of Pluricyte® Cardiomyocytes, and the potential of these cells to study cardiotoxicity, the cells were analysed by FACS, as well as by electrophysiological and impedance measurements on different MEA platforms. FACS showed high expression of the ventricular marker MLC2V (>70%) and the cardiac marker TNNT2 (>90%). Furthermore, MEA analysis showed field potentials with a pronounced repolarization peak. This was in line with simulations we performed to predict the cardiac field potential from action potential signals of adult ventricular cardiomyocytes. Field potential and impedance analysis of the effects of a range of cardioactive compounds (e.g. E4031, diltiazem, nifedipine, flecainide, isoproterenol, blebbistatin and a set of tyrosine kinase inhibitors) showed relevant pharmacological responses of the Pluricyte® Cardiomyocytes. We conclude that Pluricyte® Cardiomyocytes cultured in PCM show improved maturity, and provide a highly relevant *in vitro* model to study cardiotoxicity and efficacy of compounds at an early stage of drug development. [1] Ribeiro et al., Biomaterials, 2015.

PS 1831 Overexpression of CXCR7 Enhances EPC-Mediated Angiogenesis in Diabetic Hind Limb Ischemia through Activating Nrf2 Signal Pathway

X. Dai[2], J. Zeng[2], X. Yan[2], J. Chen[2], J. Chen[2], Y. Li[1] and Y. Tan[2]. *[1]Department of Surgery, University of Louisville, Louisville, KY and [2]KCHRI, Department of Pediatrics of the University of Louisville School of Medicine, Louisville, KY.*

Accumulating evidence shows that circulating EPCs are reduced in number and impaired in function in diabetic patients and animals. We previously found that CXCR7 is essential for endothelial progenitor cells (EPCs) survival and angiogenesis, but the mechanisms are not clear. In this study, we hypothesized that enhancement of CXCR7 expression can improve the angiogenic function of EPCs in type 2 diabetes. To test our hypothesis, EPCs were transfected with lentivirus carrying CXCR7 (Lv-CXCR7) or control lentivirus vector (Lv-null), and the angiogenic function was evaluated *in vitro* and *in vivo*. The expression of CXCR7 was evaluated under ox-LDL-mimicked diabetic conditions. We found that ox-LDL dose-dependently decreased CXCR7 expression, but without significant effects on CXCR4 expression in normal EPCs. Treatment of Lv-null EPCs with ox-LDL induced remarkable increases in oxidative stress, apoptosis and impairment of tube formation. These were mildly prevented by stromal cell-derived factor 1 (SDF-1) pretreatment while ox-LDL-induced pathological changes were almost completely prevented by SDF-1 pretreatment in Lv-CXCR7 EPCs. The angiogenic function of the engineered EPCs was also evaluated in a hind limb ischemia model in db/db mice. The results demonstrated that EPC transplantation enhanced blood reperfusion and angiogenesis in the ischemic hind limb, and the enhancement of Lv-CXCR7-EPCs was significantly greater than that of Lv-null-EPCs. Meanwhile, Lv-CXCR7-EPCs transplantation significantly upregulated proangiogenic factors (VEGF and SDF-1) and antioxidant genes (heme oxygenase-1 and quinone oxidoreductase-1) expression. Further mechanistic study found that ox-LDL inhibited the phosphorylation of Akt, GSK-3β and nuclear factor erythroid 2-related factor 2 (Nrf2) activation (nuclear translocation), which were completely prevented by CXCR7 overexpression. Moreover, CXCR7 augmented Nrf2 activation and its downstream signaling could be blocked by PI3K inhibitor. These results suggest that EPCs with high CXCR7 expression are resistant to diabetes-induced oxidative damage and have better therapeutic efficacy for hind limb ischemia in diabetes than normal EPCs, which are most likely mediated by Nrf2 activation via Akt/GSK-3β pathway.

PS 1832 A Kit to Efficiently Generate Stem Cell-Derived Hepatocyte-Like Cells for High Throughput Toxicity Screening

J. Aho, A. DeLaForest and M. Andersen. *Stem Cell, Bio-Techne, Minneapolis, MN.* Sponsor: J. Azcona-Olivera.

Secondary liver toxicity is a leading cause of commercial pharmaceutical drug recalls and compound failures during drug development. Primary hepatocytes, the most common *in vitro* model for drug-induced liver toxicity testing, are not ideal for high throughput screening due to their limited availability, difficulty to culture, and functional instability *in vitro*. Hepatocyte-like cells, derived from human pluripotent stem cells, are emerging as a stable and renewable model for drug-induced liver toxicity testing. Developing standardized differentiation protocols that reliably generate functional hepatocytes is important for the future use of stem cell-derived hepatocytes for toxicology and drug discovery. In this study, we demonstrate the reliability and efficiency our StemXVivo Hepatocyte Differentiation Kit, which uses a standardized protocol

with high quality reagents to generate functional hepatocyte-like cells from human pluripotent stem cells. The StemXVivo Hepatocyte Differentiation kit yielded a highly enriched population of pluripotent stem cell-derived hepatocyte-like cells that were functionally characterized by staining for lipid and glycogen storage, quantitative analysis of albumin and urea secretion, and assessment of p450 activity. Kit-derived hepatocyte-like cells were evaluated for use as a high throughput *in vitro* model for liver toxicity by assessing their response to known hepatotoxic molecules using a high content imaging assay. These results demonstrate that the StemXVivo Hepatocyte Differentiation Kit provides a reliable and renewable source of hepatocyte-like cells that can be utilized for high throughput toxicology and drug discovery.

PS 1833 An *Ex Vivo* Platform to Evaluate Compound Effects on Erythroid and Megakaryocyte Development

E. Clarke and G. DosSantos. *Reachbio LLC, Seattle, WA.*

Drug induced severe anemia and thrombocytopenia may be the result of drug effects on various stages of cell differentiation. The drugs may have effects on primitive precursors, lineage commitment or on the maturation of functional blood cells. We previously demonstrated the ability to assess megakaryocyte and platelet development from bone marrow (BM) derived CD34+ cells and identified drugs which had effects on various stages of maturation (SOT 2015). We extended this study to assess erythroid cell development by culturing them with SCF and Epo. We successfully showed kinetic increases in Glycophorin A (CD235a) expression over a 14 day period. A limitation in the application of these platforms is the number of CD34+ cells required. The average yield from a cord blood (CB) is approximately 1 x 10^6 CD34+ cells and may be 10 times higher from BM with a starting volume of 100 mL. Therefore multiple experiments may rarely be performed on the same donor material and for some experiments, pooled CD34+ cells are required. We reasoned that, if we could successfully expand CD34+ cells, we could assess the kinetic profiles of each lineage and have a constant supply of pre-qualified cells for comparative analyses of new drug candidates. CD34+ cell expansion was performed with a modified protocol (Boitano et al, Science August 2010) and yielded a 354 fold increase. We compared the megakaryocytes (CD41) and erythroid (CD235a) cells from CD34+ CB (purity of 91.3%), CD34+ BM (purity of 98.0%) and from cells expanded as described (purity of 94.5%). At 14 days culture in lineage specific media formulations, the profile of the resulting cells from the expanded cell population was more like that of blood. The percentage of CD45- CD235a+ cells was 23-29% when CB or BM CD34+ cells were used as the starting cell population, but 70% when the starting cell population was expanded CB CD34+ cells. This platform can provide more consistency in donor selection, allow for a larger scale evaluation of drug candidates while maintaining the integrity of assessing mechanistic effects on blood cell development.

PS 1834 Cadmium and Arsenic Transformed Human Peripheral Lung Cells Expressing Cd34 Display Stem Cell-Like and Malignant Properties

N. Makia, N. Ngalame and E. J. Tokar. *NTP, NIEHS, Durham, NC.*

Lung cancer is the leading cause of cancer-related death worldwide. The lung is a human target for cadmium and arsenic carcinogenesis. Previous studies showed that human peripheral lung (HPL-1D) cells acquired malignant phenotype at weeks 20 and 38 after chronic cadmium (5 μM) and arsenic (2 μM) exposure, respectively. Here, we hypothesize that the cancer phenotype of the transformed HPL-1D cells is maintained by cancer stem cells (CSCs) that possess SC-like properties. CSCs express tissue-specific cell surface markers which are used for their identification and isolation such as CD44, CD133 and CD34. Flow cytometry analysis of HPL-1D showed putative SCs/CSCs express CD34 at 2.5%. CD34+ cells were successfully isolated with ≈ 90% purity using an autoMACS magnetic bead separator. These cells showed enhanced expression of pluripotency-associated genes (CD34, NANOG, SOX2 and ALDH1A1) compared to the total population. Holoclones are tightly-packed cell clusters often enriched in SCs/CSCs. Compared with putative SCs, arsenic-induced CSCs formed 2-fold more holoclones while cadmium CSCs showed a decrease in holoclone numbers, suggesting that SCs may be sensitive to cadmium cytotoxicity but possess a survival advantage to arsenic. Compared with control SCs, the cadmium and arsenic CSCs hyper-secreted matrix metalloproteinase-2 and showed high levels of cell cycle regulator cyclin D1, both characteristics of cancer cells. PTEN (transcript and protein) and p16 (transcript) were decreased in cadmium or arsenic CSCs indicating depletion of tumor suppressor genes in these cells. Increased vimentin and decreased E-cadherin expression provided evidence of epithelial-mesenchymal transition, a common occurrence during acquisition of cancer phenotype. These data indicate that CD34+ cells isolated from cadmium or arsenic-transformed HPL-1D cells possess a CSC-like phenotype. This study may help in the development of effective therapy for the treatment of lung cancer by targeting CSCs.

PS 1835 Aluminum Maltolate Alters Cell Cycle Progression in Mouse Embryonic Stem Cells

A. Aliberti and F. A. Barile. *Department of Pharmaceutical Sciences, St. John's University, Queens, NY.*

Prenatal exposure to environmental pollutants disrupts developmental programming, leading to the establishment of disease phenotypes. Environmental pollutants with this potential include metals, such as aluminum (Al). Using undifferentiated mouse embryonic stem (mES) cells as a model of embryogenesis, the effects of aluminum maltolate (AlMal) and aluminum chloride (AlCl3) were evaluated on cytotoxicity, cell cycle progression, and gene expression of pluripotency markers. Exposure to AlMal (120-200 μM for 24 hours) caused a statistically significant increase in cytotoxicity as determined by LDH release assay, while exposure to AlCl3 (40-160 μM) caused a significant decrease in cytotoxicity as compared to control. Cell cycle analysis revealed that 150 and 300 μM AlMal (IC25 and IC50, respectively) resulted in an increase in percentage of cells in the G2/M-phase as compared to control. Additionally, there was a significant decrease in the percentage of cells in S-phase following exposure to both concentrations, while a decrease in G0/G1-phases was observed following 150 μM only. Higher concentrations of AlCl3 (150 and 300 μM), however, did not change the percentage of cells in any phase of the cell cycle. AlMal (300 μM) revealed a greater than 2-fold down-regulation of Sox2, but no alteration of expression of Pou5f1 and Nanog. The ability of AlMal to disrupt normal cell cycle progression and interfere with gene expression of pluripotency transcription factors suggests the potential to disrupt processes involving normal embryonic development.

PS 1836 Exposure to Fine Airborne Particulate Matter (PM2.5) Impairs Bone Marrow-Derived Endothelial Progenitor Cell Function

T. O'Toole, W. Abplanalp, D. Conklin, P. Haberzettl and A. Bhatnagar. *University of Louisville, Louisville, KY.*

Exposure to fine airborne particulate matter (PM2.5) is associated with cardiovascular disorders but the basis for this is not entirely clear. We have previously shown that humans and mice exposed to PM2.5 have decreased levels of circulating endothelial progenitor cells (EPCs) that may be attributed to a bone marrow mobilization defect. A deficit of these reparative cells may contribute to the development of endothelial dysfunction and thus be an underlying mechanism contributing to the origin and progression of atherosclerosis, insulin resistance, hypertension, and impaired wound healing. However whether PM2.5 exposure also alters the functional capacity of EPCs is not known. Here we exposed C57BL/6 mice to filtered air or concentrated ambient particles (CAPSs) for 6h per day for 9 days, and then cultured bone marrow-derived EPCs. We determined that EPCs from CAPs-exposed mice exhibited defects in mitochondrial function, proliferation and *in vitro* tube formation compared to comparable cells from mice exposed to filtered air. Using a model of hind limb ischemia, we also showed that these CAPs-derived cells were less efficient in re-establishing vascular perfusion than cells from filtered air-exposed mice. These results demonstrate that PM2.5 exposure induces functional defects in vascular reparative EPCs. The combined quantitative and qualitative effects of PM2.5 on EPCs may underlie the cardiovascular pathologies associated with air pollution exposure.

1837 Prenatal Cigarette Smoke (CS) Exposure Causes Alteration in Hematopoietic Stem and Progenitor Cells (HSPCs) and Epigenetic Modifications

K. P. Singh[3], N. Englert[1], Z. Unnisa[3], J. A. Bennett[3], E. Henry[3], T. Thatcher[3], B. Bennett[1], M. R. Campbell[1], P. J. Sime[3], R. P. Phipps[3], T. A. Gasiewicz[3] and D. A. Bell[1]. *[1]NIEHS, Research Triangle Park, NC; [2]School of Medicine and Dentistry, University of Rochester, Rochester, NY and [3]Environmental Medicine, School of Medicine and Dentistry, University of Rochester, Rochester, NY.* Sponsor: K. Singh.

Prenatal CS exposure has been associated with many diseases in children and adults, including cancers such as lymphoma and leukemia, but the underlying mechanism is still unknown. Recently, differential DNA methylation of Gfi1, Cyp1a1, and Ahrr, were found in cord blood DNA from newborns with *in utero* tobacco smoke exposure. Gfi1 and Ah Receptor (AhR) are key regulators of hematopoiesis and HSPCs, while Cyp1a1 and AhR repressor (Ahrr) are regulated via activation of AhR. CS contains numerous AhR agonists that can cause persistent activation of the AhR pathway. We hypothesized that prenatal CS exposure may alter DNA methylation in developing embryo HSPCs that could affect susceptibility to hematopoietic diseases later in life. Pregnant female C57BL/6 mice were exposed to CS (40 mg/m3 for 3h/day, 5 days/week) from embryonic days 0 to 18. Female pups were evaluated at 4 weeks of age. In smoke-exposed pups, liver and kidney weights were significantly increased. Total white blood cells, monocyte, granulocyte and platelet numbers were significantly decreased in these pups. In bone marrow (BM), Gr-1+, Mac-1+ and Ter-119+ cells were significantly decreased while BM stem/progenitor LSK cells were increased. Spleen contained significantly decreased B220+ cells. To identify differentially methylated regions (DMRs), DNA from BM HSPCs (Lin-) isolated from prenatally smoke-exposed pups was analyzed by reduced representation bisulfite sequencing. Significant DMRs were observed in or near many genes involved in hematopoiesis, hematopoietic disease and cancers, particularly the Hoxa gene cluster, Ptpn1, Ptprs, Mafg, Pdgfa, Tfr2 and Meis. Our preliminary findings suggest that prenatal CS exposure can produce epigenetic changes in HSPCs that may be associated with susceptibility to hematopoietic disease later in life. (Funded by ES01247, ES07026, and ES04862).

1838 Deceleration of Cell Cycle of Primitive Hematopoietic Progenitors (CFU-S13) from Senescent Mice at Steady State Was Reactively Accelerated After 2-Gy Whole-Body Irradiation

Y. Hirabayashi[4], I. Tsuboi[2], K. Kurata[1], J. Kanno[4] and Y. Kusunoki[3]. *[1]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; [2]Department of Functional Morphology, Nihon University School of Medicine, Tokyo, Japan; [3]Department of Radiobiology/ Molecular Epidemiology, Radiation Effects Research Foundation, Hiroshima, Japan and [4]Division of Cellular and Molecular Toxicology, Center for Biological Safety and Research, National Institute of Health Sciences, Tokyo, Japan.*

Two-Gy whole-body irradiation (WB-IR) revealed that mature hematopoietic progenitor cells (HPCs) in aged mice could provide progenies only during steady-state hematopoiesis but lose the additional capability to proliferate owing to WB-IR. Moreover, primitive HPCs [colony forming unit in spleen (CFU-S) on day 13] in aged mice showed a decelerated cell cycle during the steady state but showed an accelerated response after recovery from damage caused by WB-IR. In this study, various cell-cycle parameters of colony-forming HPCs [CFU-granulocyte/ macrophage (GM), CFU-S on day 9 (CFU-S9), and CFU-S13 for mature, less mature, and immature HPCs, respectively] were evaluated by bromodeoxyuridine (BrdUrd)-ultraviolet (UV)-A assay in young and aged mice (6 weeks and 21 months of age, respectively) with or without 2-Gy WB-IR after a 4-week recovery period when all the peripheral blood parameters had recovered. As a result, to compare the cell kinetics among all types of HPC in the young and aged groups with or without 2-Gy WB-IR, 1) the percent incorporation of BrdUrd at time zero of all types of HPC in young groups was significantly high after WB-IR, although those in the aged groups were not significantly different. 2) The generation doubling time of all types of HPC was increased by WB-IR in both young and aged groups, except that of CFU-S13 in aged mice. 3) Only CFU-S13 in the WB-IR aged group showed a significant increase in the cumulative cycling cell fraction. This suggests that the mechanisms of peripheral blood recovery after 2-Gy WB-IR are markedly different between young and aged mice. Thus, primitive HPCs, i.e., CFU-S13, decelerate their cell cycle in the steady state during aging, significantly respond to WB-IR, and recover in number of peripheral mature blood cells to the stable level. (This study was supported in part by HHSN272200900059C)

1839 Epigenetic Mechanisms of Early Germ Cells Sensitivity to Environmental Chemicals

J. Camacho, Z. Lundby, A. Clark and P. Allard. *UCLA, Los Angeles, CA.*

Germ cells are the bridge between generations and their integrity is paramount to the health and viability of all organisms. As such, the dysregulation of germ cells function significantly contributes to infertility and is also the leading cause of birth defects and infant deaths in the United States. Remarkably, although there is a clear contribution of environmental chemicals to the etiology of human germ cell errors, their mechanisms of action have remained elusive. Here, we examine the significance of environmental perturbations on the epigenome of germ cells. We hypothesize that as embryonic germ cells undergo an extensive remodeling of their chromatin, including genome-wide demethylation, they are particularly sensitive to environmental exposures. To test this hypothesis, we have established a dual model approach including the use of the genetic model system Caenorhabditis elegans and *in vitro* derived mouse Primordial Germ Cell-Like Cells (PGCLCs). First, we are making use of a worm strain where GFP is specifically epigenetically silenced in the germline. We show that valproic acid, a well-known mammalian histone deacetylase inhibitor, disrupts the germline epigenetic state leading to the de-silencing of the transgene in the germline. Our current experiments test this concept further by exposing the worms to two environmental compounds: Vinclozolin and BPA and analyze a disruption in maintenance and/or establishment of epigenetic marks. We show that both chemicals cause a strong de-silencing effect that lasts for at least three generations. Furthermore, most worms showing de-silencing in the third generation originate from worms showing de-silencing in earlier exposed generation indicating that the effect is inherited. We also present preliminary results in PGCLCs demonstrating that this new germ cell model is applicable to chemical testing. Together, we show that Vinclozolin, and BPA disrupt the germline epigenome over several generations in a heritable fashion. These results offer a more comprehensive view of environmental effects on the epigenome through multiple generations.

1840 Long-Lasting Cognitive Deficits in Rhesus Monkeys After Neonatal General Anesthesia Induced by Sevoflurane

M. G. Paule[1], M. Li[1], X. Zhang[1], S. Liu[1], J. P. Hanig[2], W. Slikker[1] and C. Wang[1]. *[1]Division of Neurotoxicology, National Center for Toxicological Research, US FDA, Jefferson, AR and [2]Office of Testing and Research, Center for Drug Evaluation and Research, US FDA, Silver Spring, MD.*

It was shown previously that general anesthesia induced by 24 hours of ketamine or 8 hours of nitrous oxide/isoflurane during the first week of life causes significant increases in neuronal cell death and permanent cognitive deficits in rhesus monkeys. In the present study, 5 monkeys were exposed on PND 5 or 6 to sevoflurane (sevo, 2.5 % in oxygen) anesthesia (maintenance of a light surgical plane) for 8 hrs. Ten control animals were unexposed. At 7 months of age all animals began training to perform a series of cognitive function tasks as part of the National Center for Toxicological Research (NCTR) Operant Test Battery (OTB). Tasks included those for assessing aspects of learning, motivation, color discrimination, and short-term memory. Subjects responded for banana-flavored food pellets by pressing response levers and press-plates during daily (M-F) test sessions (50 min) and were assigned training scores based upon their individual task performance. Beginning almost at the outset of training sevo-exposed animals responded more slowly in all tasks and less accurately in the learning task. In addition, sevo-exposed animals also mastered the concept of 'matching' in the short-term memory task much more slowly than controls. These effects have remained apparent for almost a year of testing (up to the present). These long-term cognitive impairments seen after an 8hr sevo exposure appear to be even more severe than those noted in our previous ketamine and isoflurane/nitrous oxide studies and provide additional evidence that general anesthesia of sufficient duration and occurring during a sensitive period of brain development, can result in very long-lasting deficits in brain function in primates. Supported by NCTR and CDER of the US FDA.

1841 Embryonic Iron Deficiency Permanently Alters Neural Structure: A Mechanism to Explain Some of the Long Term Consequences of Early Life Iron Deficiency

M. Rudy and M. Mayer-Proschel. *University of Rochester, Rochester, NY.*

Iron deficiency (ID) is the most common micronutrient deficiency, affecting more than nine percent of reproductive age women in the United States and over a billion women worldwide. When an iron deficient woman becomes pregnant, her body cannot provide the iron a developing fetus requires, and if low iron occurs during critical windows of development, there is an increased risk of psychiatric disorders, behavioral problems, and learning difficulties. Despite an awareness of this risk, the developmental mechanism linking embryonic iron availability to these adult diseases, remains largely unknown. We established a mouse model of gestational ID where the mother mouse is given a diet with adequate iron to meet her daily needs but too little to support the increased iron requirement during pregnancy. As is often seen in humans, this model produces mild anemia during the third trimester which is then treated with postnatal iron supplementation. We found that adult mice, which were gestationally iron deficient, had a significantly impaired response to both a GABA antagonist and a glycine agonist. These mice had an increased number of inhibitory GABAergic interneurons in the cerebral cortex, with no apparent decrease in excitatory pyramidal neurons. We also examined the region which gives rise to GABAergic interneurons during embryonic development and found an expansion of both Nkx2.1 and Gli1 signaling: two factors which are important for appropriate specification of GABAergic interneurons. Taken together, our data suggests that embryonic ID increases inhibitory signaling in the adult brain by expanding the region which produces inhibitory GABAergic interneurons during development, thereby increasing the number of inhibitory interneurons in the adult cortex. Furthermore, we hypothesize that this imbalance between excitation and inhibition within the brains of a gestationally iron deficient individuals would shift the dose response curve to certain drugs such as benzodiazepines and ethanol, in such a way that affected individuals are more sensitive.

1842 Developmental Toxicant Exposure and Early Neuroimmune Susceptibility to Alzheimer's Disease

A. vonderEmbse, Z. W. Hinton, Q. Hu and J. C. DeWitt. *Pharmacology and Toxicology, East Carolina University Brody School of Medicine, Greenville, NC.* Sponsor: J. DeWitt.

The role of developmental immune dysfunction in neurodegenerative diseases, such as Alzheimer's disease (AD), may be due to an early disruption of regulatory signals for activation of microglia, the resident macrophage of the central nervous system (CNS). Although microglia are thought to contribute to AD pathology by perpetuating chronic inflammation, there are data to suggest pathological contribution is due to a decrease in neuroprotective capabilities rather than neurotoxicity. We previously demonstrated that a "double-hit" model of postnatal lead (Pb) exposure in a triple transgenic mouse model for AD (3xTgAD) lead to microglial activation abnormalities and exacerbated late-stage AD pathologies. Here, we included a wildtype (WT) comparison and investigated the persistence and dysregulation of microglial activation states following toxicant exposure from postnatal day (PND) 5-10. Surface markers for classical, pro-inflammatory M1 activation (CD86) and alternative, anti-inflammatory M2 activation (CD209) were analyzed at PND 120, 180, and 240, with greater pro-inflammatory markers at the earliest timepoint (PND 120) and lower anti-inflammatory markers following postnatal Pb exposure. Furthermore, double-positive (M1+/M2+) stained microglia, indicative of dysregulated activation, increased with both time and Pb exposure regardless of genetic predisposition. Taken together, these data suggest regulation of microglia activation is perturbed during development due to toxicant exposure, which then persists into adulthood and impairs neuroprotection via an initial decrease in M2 surface markers and aberrant activation profiles at later stages of pathogenesis. Further investigation of the mechanisms by which regulation of microglial activation is altered following an early immune insult will provide critical insight into the precarious developing neuroimmune system and its potential role in AD etiopathology.

1843 Early-Life Toxicant Exposure and Markers of Neuropathology in a Model of Alzheimer's Disease

Q. Hu, C. Boles, A. vonderEmbse and J. DeWitt. *East Carolina University, Greenville, NC.*

Alzheimer's disease (AD) is a form of dementia that gradually worsens over time, affecting memory, thinking, and behavior. The disease is normally a late onset neurodegenerative disorder; however increasing evidence suggests that early-life changes in brain development could precipitate earlier onset or changes in progression, especially in a genetically susceptible model. Our hypothesis is that early-life exposure to toxicant will reprogram microglia, change their responsiveness during the aging process, and alter the onset or progression of the disease in a susceptible model. Pregnant triple transgenic mice (3xTgAD) were given 100 mg/kg of lead acetate or a vehicle control from gestational days 7-11, a critical period for development of microglia. Wild-type (WT) mice were similarly exposed. Litter outcomes, including litter weights, litter size, and sex ratios, did not differ by lead treatment. Offspring were weaned at postnatal day (PND) 21 and were evaluated for various endpoints at PND 50, 90, and 180 to determine if the combination of genetic vulnerability, early-life exposure to an exogenous agent, and aging exacerbated of neuropathological markers indicative of AD. In both male and female 3xTgAD offspring exposed to lead prenatally, brain concentrations of amyloid beta-42 were higher than in control 3xTgAD mice and in treated WT mice at PND180; amyloid beta plaque size also was greater in the exposed 3xTgAD mice. In all groups, when evaluated by flow cytometry and with immunohistochemistry, the total number of amoeboid microglia decreased with age but the ratio of amoeboid to ramified microglia favored amoeboid microglia. However, in 180 day old exposed 3xTgAD mice, the number of amoeboid microglia and the ratio of amoeboid to ramified microglia was decreased relative to control 3xTgAD mice and in treated WT mice. One hypothesis that could arise from these data is that the neuroprotective capabilities of microglia are diminished by early-life disturbances in a genetically susceptible model, thus favoring amyloid beta accumulation. Although this is contrary to many hypotheses that support a neuroinflammatory and neurotoxic role for microglia, our data suggest that a reduction in the number of "active" microglia is associated with neuropathology. Current work in our lab is exploring this hypothesis in an AD model.

1844 Investigating the Role of the Epicardium in Heart Development: Insights from the *EHC* Mouse

L. Ridge[1], G. Tenin[1], E. Barnes[2], J. Wright[2] and K. Hentges[1]. [1]*Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom and* [2]*Syngenta, Jealott's Hill International Research Centre, Bracknell, United Kingdom.* Sponsor: S. Plummer.

Congenital heart defects cause extensive morbidity and pose a global health and economic burden. Although causes are largely unknown, agrochemical toxicology studies have demonstrated a link between certain compound chemistry and the formation of double outlet right ventricle (DORV) and ventricular septal defects (VSD). Developmental biology approaches that investigate the processes underpinning these conditions, offer unique insights that may simultaneously inform the design of novel therapies to repair the injured heart, and improve agrochemical product safety. We have generated a mouse model, called EHC, that displays DORV, VSD and defective coronary vessel development. EHC mutants harbour a recessive embryonic lethal mutation in Myh10, resulting in the loss of non-muscle myosin IIB (NMIIB). We aim to elucidate the role of NMIIB in heart development. During cardiogenesis, a subset of cells within the epicardium, the heart's outer surface, undergo epithelial-mesenchymal-transition (EMT) to give rise to epicardial-derived cells (EPDCs). EPDCs invade the underlying myocardium and differentiate into vascular smooth muscle that is essential for the correct formation of the coronary vasculature. EPDCs also provide crucial signals for myocardial proliferation and functionality. However, the molecular mechanisms that regulate epicardial biology remain unclear. Using a range of immunohistochemistry (IHC) and imaging techniques, we show that EHC epicardial cells display abnormal morphology and behaviour. We employed Wilms Tumour 1 IHC on paraformaldehyde fixed embryonic heart sections to track EHC EPDCs and establish that their migration into the myocardium is significantly impaired. Moreover, IHC analysis of markers for EMT (Snail), and proliferation (phosphohistone H3), indicates that EHC epicardial cells exhibit reduced EMT. These results reveal a novel role for NMIIB in facilitating both epicardial EMT and subsequent EPDC invasion. Future work will utilise an *in vitro* epicardial culture system to identify signalling pathways that require NMIIB function to instigate EMT. Together, this research will help dissect the dysfunctional mechanisms that contribute to abnormal heart development.

1845 DNA Methyltransferase 3a Is a Possible Mediator of *In Utero* Caffeine Effects on Adult Cardiac Function

X. Fang[2], R. Poulsen[2], J. Wang-Hu[2], N. S. Calvo[2], C. S. Simmons[2], S. A. Rivkees[2] and C. C. Wendler[2]. [1]*Mechanical & Aerospace Engineering, University of Florida, Gainesville, FL and* [2]*Pediatrics, University of Florida, Gainesville, FL.*

Our previous research demonstrated that *in utero* caffeine exposure at 20 mg/kg in mice at embryonic days (E) 6.5-10.5 decreases the expression of DNA methyltransferases (DNMTs) in embryonic ventricles, induces both short-term and long-term changes in cardiac gene expression and DNA methylation patterns, and decreases adult cardiac function. We hypothesize that these *in utero* caffeine effects on cardiac function are mediated by down-regulation of DNMTs. To test this hypothesis, Dnmt1, Dnmt3a, and Dnmt3b siRNAs were used to reduce DNMT expression in primary embryonic mouse cardiomyocytes. At 24 h post-transfection, knock down of DNMT1, 3a, and 3b markedly increased cytotoxicity and apoptosis. Reduced DNMT3a expression disrupted sarcomere assembly and altered mitochondrial morphology, as assessed by immunostaining and electron microscopy. Furthermore, down-regulation of DNMT3a decreased the beating frequency of the naturally contracting cardiomyocytes. In addition, reduced contractile movement, reduced velocity of contraction and relaxation, and variability in the time between contractions were observed in the Dnmt3a siRNA treated cells using a video-based imaging assay. Fluo-4 Direct Calcium assay showed that down-regulation of DNMT3a reduced cytosolic calcium signaling. DNMT3a deficient cells had reduced expression of cardiac genes that are involved in dilated cardiomyopathy, including Myh6, Myh7, Tnnc1, Tnnt2, Tnnti3, Bnp, and Nfatc1. This is consistent with the weakened contractility in DNMT3a deficient cells. In contrast, Dnmt1 and 3b siRNA treatments had no adverse effects on cardiomyocyte morphology or function. Collectively, these data indicate that disruption of DNMT3a activity has direct effects on cardiomyocyte gene expression, cellular structure, and function, possibly mediating the *in utero* caffeine effects on adult cardiac function.

1846 Maternal PM2.5 Gestational Exposure Alters Fetal Size, Cardiac Function, Placental Morphology, and Postnatal Growth in Mice

K. Kaur[1], C. Phoon[3], M. Veras[2], J. Blum[1], S. Doherty-Lyons[1], P. Tijerina[1], S. Attreed[1], L.-C. Chen[1] and J. Zelikoff[1]. [1]*Env Med, NYU, Tuxedo, NY;* [2]*Pathology, U of São Paulo, São Paulo, Brazil and* [3]*Pediatrics Cardiology, NYU, New York, NY.*

Epidemiologic research shows a causal link between PM2.5 exposure and CVD in adults, while fetal CVD outcomes are less explored. To examine if maternal gestational exposure to inhaled PM impacts fetal size, cardiac function, placentation and postnatal growth, pregnant B6C3F1 mice were exposed (6hr/d) to either filtered air (FA; 4µg/m3) or concentrated ambient PM (CAPs; 113µg/m3) from Tuxedo, NY from either gestational day (GD) 0.5-16.5 (Window 1; W1) or only during organogenesis GD6.5-14.5 (Window 2; W2). Fetal crown-to-rump length (CRL), fractional area change (FAC), intracardiac and umbilical cord Doppler waveforms were measured by ultrasound-biomicroscopy (UBM) on GD12.5. Dams exposed during W1 were euthanized on GD17.5, CRL measured and placentas collected for stereology; at PND21 offspring BW was assessed and hearts collected for stereology. Building on our previous data showing PM-induced decreases in fetal FAC (W1) and CRL (W2) on GD12.5 and reduced in offspring BW on GD17.5 (W1) and PND21 (W2), recent studies using the same mice extend our findings revealing that maternal CAPs exposure: prolongs fetal isovolumic contraction time (32.8ms vs. control 17.1ms) in W1; decreases umbilical artery peak velocity (69.5mm/s vs. control 82.3mm/s) in W2; alters labyrinth zone morphology of W1 CAPs-exposed GD17.5 placentas revealing a 26% increase in fetal capillary (FC) surface area density, and a 33% and 25% decrease in FC total volume and surface area ratio between the FC and maternal blood spaces, respectively. At PND21, cardiac stereology revealed a 12% increase in left ventricle volume fraction, a 38% decrease in left atrium total volume in W2 and a 30% increase in cardiomyocyte volume in W1 CAPs-exposed offspring vs. control. This study suggests that some cardiac pathologies linked with PM exposure in adults may actually begin *in utero*. Grants: March of Dimes, NIEHS ES000260.

1847 Apparent Sex-Differences and Immune Alterations Due to Prenatal and Postnatal Exposure to Concentrated Ambient Particulate Matter

P. B. Tijerina, J. L. Blum, D. Lauterstein, G. Grunig, L.-C. Chen and J. T. Zelikoff. *Environmental Medicine, NYU School of Medicine, Tuxedo, NY.*

Approximately 3.7 million premature deaths annually are linked to ambient air pollution, and among these deaths 127,000 are children under the age of 5 years. An increasing number of immune-related disorders (e.g. asthma) have been associated with exposure to elevated levels of particulate matter (PM) during early life stages. To evaluate this vulnerable exposure period, timed-pregnancies in B6C3F1 mice were conducted and dams/neonates were exposed throughout gestation (6 hr/d) and during a 10-day postnatal period (2 hr/d) to either concentrated fine-sized (≤ 2.5 um) ambient particulate matter (CAPs) or filtered air (FA). Gravimetric analyses revealed daily mean CAPs concentrations of 126 ug/m3 and 162 ug/m3 for pre- and post-natal exposures, respectively. At 6- and 15-wk-of-age immune parameters were assessed in male and female offspring, including serum cytokine levels and flow cytometry. A model of ovalbumin (OVA)-induced allergic asthma was also conducted using a subset of adult offspring in which airway inflammation and hyperresponsiveness (AHR) were evaluated. Preliminary data revealed circulating levels of IL-17A were significantly elevated in CAPs-exposed female offspring compared to their sex-matched FA counterparts. In contrast, serum IFN-γ was elevated and IL-10 was reduced only in CAPs-exposed male offspring. Additional immune cell profiling performed on splenocytes showed a significant reduction in Foxp3 expression in CAPs-exposed female offspring. These apparent sex-differences highlight the importance of evaluating both sexes in developmental immunotoxicity studies. Data from this study demonstrates the negative impacts of PM exposure on the developing immune system, proposing critical consequences for offspring exposed during early life stages. These findings pose a global threat to current and future generations that may have lasting consequences and should be considered when assessing public health risk in vulnerable populations. Supported by funding from March of Dimes and NIEHS center grant ES000260.

1848 Aryl Hydrocarbon Receptor Dependent Mitochondrial Toxicity of PAHs

N. Jayasundara[3], J. S. Kozal[3], A. J. Bone[3], C. D. Lindberg[3], J. Bailey[3], S. Burwell[1], E. D. Levin[2,3], J. N. Meyer[3] and R. T. Di Giulio[3]. [1]*McGill University, Montréal, QC, Canada;* [2]*Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC and* [3]*Nicholas School of the Environment, Duke University, Durham, NC.*

Mitochondrial dysfunction is associated with numerous diseases. Environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs), are known to impair mitochondrial function. However, the mechanisms underlying mitochondrial toxicity PAHs are poorly understood. Here, we examined mitochondrial effects of non-teratogenic exposures to simple and complex PAH mixtures and the role of aryl hydrocarbon receptor (AHR), a key pathway involved in xenobiotic metabolism, in mediating these effects in zebrafish, *Danio rerio*. We exposed zebrafish embryos to a mixture of benzo(a)pyrene + fluoranthene or to a complex PAH mixture derived from a sediment extract from a highly PAH contaminated Superfund site. We conducted these experiments in control and AHR morphant embryos to evaluate the role of this receptor in mediating PAH toxicity and characterized embryonic mtDNA copy number and damage and mitochondrial function following exposure. To assess if developmental bioenergetic impairment persists later in life we examined heart and brain mitochondrial function *ex vivo* using a novel method based on Seahorse extracellular flux analyzer. To determine the relationship between mitochondrial, tissue, and whole organism bioenergetics, we also evaluated swimming performance, aerobic respiration, and neurobehavioral activities. Results show that early life exposure to PAH mixtures results in embryonic mtDNA damage, as well as AHR-dependent changes in embryonic mitochondrial function and metabolic partitioning that persist through life at the tissue and whole organismal levels. Behavioral studies demonstrate PAH induced AHR-dependent effects on larval locomotor activity and persistent alteration of adult responses to various stimuli. These findings lend to a previously presented hypothesis suggesting a putative role for AHR in regulating mitochondrial membrane potential. Overall, our results indicate that early life exposure to low levels of PAH mixtures and transient changes in AHR levels have persistent bioenergetic effects and provide mechanistic insights into mitochondrial toxicity of developmental exposure to environmental contaminants. This work is supported by NIEHS T32-ES021432 & P42ES010356.

1849 Defining the Long-Term Behavioral and Physiological Effects of Developmental Exposure to Oxygenated Polycyclic Aromatic Hydrocarbons (OPAHs) in Zebrafish

A. L. Knecht[2], L. Truong[2], S. Proffitt[2], E. Johnson[2] and R. L. Tanguay[2]. [1]*Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR and* [2]*Superfund Research Center, Oregon State University, Corvallis, OR.*

Oxygenated polycyclic aromatic hydrocarbons (OPAHs) are environmental contaminants present in urban air, dust, soil and water, resulting from incomplete combustion of organic materials or fossil fuels, and photo-oxidation of PAHs. It is widely recognized that OPAHs pose risks to human health, especially for the developing fetus and infant where developmental exposures have been linked to complex human diseases later in life. Previously, we demonstrated that developmental OPAH exposures result in altered oxygen consumption and photo-locomotor response in larval zebrafish. To determine if these effects persisted, we used the zebrafish model to evaluate the adult consequences of early developmental exposures to benzanthrone (BEZO), benzo[a] anthracene-7,12-dione (7,12-B[a]AQ), and 9,10-phenathrenequinone (9,10-PHEQ). Zebrafish embryos were continuously exposed between 6-48 hours post fertilization (hpf) to OPAH concentrations that caused no observable developmental toxicity, raised to adulthood in chemical-free water, and assessed for changes in swimming, social behavior, learning, and oxygen consumption during exercise. Fish developmentally exposed to 9,10-PHEQ and 7,12-B[a]AQ had deficiencies in learning and performance in an active avoidance conditioning test. Fish developmentally exposed to BEZO and 7,12-B[a]AQ displayed aberrant social interactions and significant changes in swimming behavior. A swim tunnel respirometer was also used to measure total oxygen consumption as a measure of adult cardiovascular fitness. Zebrafish developmentally exposed to 7,12-Ba[A]Q, BEZO and 9,10-PHEQ had significant differences in oxygen consumption rates compared to control animals. These data demonstrate that developmental OPAH exposures result in long-term adverse behavioral and physiological responses in adult animals. This research was supported by the NIEHS grant P42 ES016465 and P30 ES000210.

1850 Perinatal Exposure to the Flame Retardant Triphenyl Phosphate Accelerates Type 2 Diabetes and Causes Obesity in UCD-T2DM Rats

A. J. Green[1], J. L. Graham[3], G. Cano-Sancho[3], E. A. Gonzalez[3], P. J. Havel[3] and M. A. La Merrill[3]. [1]*Department of Biological Sciences, North Carolina State University, Raleigh, NC;* [2]*Department of Environmental Toxicology, University of California, Davis, CA and* [3]*Department of Nutrition, University of California, Davis, CA.*

Triphenyl phosphate (TPP) is a major component of the flame retardant mixture, Firemaster 550 (FM 550), which is widely used in products available throughout the United States. Analysis of household dust samples has shown TPP concentrations as high as 1800 µg/g of dust. This could have adverse health effects as developmental exposure to FM 550 resulted in an increase in body mass, altered thyroid hormones, and insulin resistance in rats. In this study we hypothesized that developmental exposure to the commercial flame retardant TPP would cause obesity and accelerated type 2 diabetes mellitus (T2DM) onset in the UCD-T2DM rat model of T2DM. We administered TPP to UCD-T2DM rats from gestational day 8.5 to postnatal day 21 (weaning) and studied their metabolic phenotypes until 3.5 or 6 months of age to assess obesity and T2DM, respectively. Perinatal TPP exposure increased the body and fat mass, leptin, and cumulative food intake of 3.5 month old rats. TPP also increased the accumulation of lipid droplets in differentiating 3T3-L1 preadipocytes. Perinatal TPP exposure more than doubled the prevalence of T2DM in 6 month old weight matched rats (79% and 33% prevalence of T2DM if perinatally exposed to TPP vs vehicle control, respectively). TPP also enhanced the basal- and insulin- stimulated uptake of glucose analog 2-NBDG in differentiated 3T3-L1 adipocytes while it reduced their sensitivity to insulin action. These observations suggest that perinatal exposure to TPP causes obesity and accelerates T2DM onset by targeting the adipocyte. We have also shown that the UCD-T2DM rat is an effective model for testing developmental exposure to environmental pollutants and their effects on diabetes onset and obesity.

1851 Obstetric Factors Affect Immune Responses at Teenage in Children Born by C-Section

M.-V. Martikainen[3], L. Keski-Nisula[1], A. Karvonen[2], J. Pekkanen[4], M.-R. Hirvonen[3] and M. Roponen[3]. [1]*Kuopio University Hospital, Kuopio, Finland;* [2]*National Institute for Health and Welfare, Kuopio, Finland;* [3]*University of Eastern Finland, Kuopio, Finland and* [4]*University of Helsinki, Helsinki, Finland.* Sponsor: M. Viluksela.

Background: Obstetric factors including birth by cesarean delivery could affect the maturation of immune system and thus modify immune responses later in life. Objective: Our aim was to investigate associations between obstetric factors and the function of innate immune system of adolescents born by cesarean section. Methods: We stimulated peripheral blood mononuclear cells of adolescents born by cesarean section with different Toll-like receptor ligands (TLR2, Peptidoglycan; TLR3, Poly(I:C); TLR4, LPS) and measured excretion of regulatory (IL-2, IL-10), inflammatory (IL-1β, TNF, IL-6), Th1 (IFN-γ, IL-12p70) and Th2 (IL-4, IL-13) associated cytokines and a chemokine (CXCL8) using a multiplexed immunoassay. Obstetric factors and allergic endpoints were assessed by questionnaires. Skin prick-tests were used to assess allergic sensitization. Amniotic fluid or swab samples were cultured to assess the intrauterine growth of microbes. Results: Advanced cervical dilation (≥6 cm) at the time of cesarean delivery associated significantly with the spontaneous production of IL-2, IL-1β, TNF, IL-6, IL-13, and CXCL8 and also with TLR3- and TLR4-stimulated TNF production compared to adolescents/children who were delivered electively or with earlier stages of labor. Treatment in neonatal intensive care unit associated with lower spontaneous as well as TLR stimulated production of cytokines at teenage. Intrauterine growth of pathogens at the time of birth was associated with TLR3-stimulated IL-13 at teenage. Conclusions: Our novel data suggest that obstetric factors have long-term effects on immune function among children born by cesarean delivery.

1852 Fatty Liver Increases Gastro-Intestinal Toxicity by Modulating TLR-4 Trafficking into Lipid Rafts

V. Chandrashekaran[3], S. Das[3], D. Dattaroy[3], F. Alhasson[3], M. M. O. Pena[3], J. Carson[3], F. Berger[3] and S. Chatterjee[3]. [1]*Center for Colon Cancer Research, University of South Carolina, Columbia, SC;* [2]*ENHS, University of South Carolina, Columbia, SC and* [3]*Excercise Science, University of South Carolina, Columbia, SC.* Sponsor: S. Chatterjee.

Obesity is well associated with the development of fatty liver disease and colorectal neoplasms. Interestingly recent clinical studies found a strong association of colon inflammation and toxicity with fatty liver disease. We hypothesise that oxidative stress due to NADPH oxidase is responsible for Calcium influx via Per-oxynitrite formation resulting in TLR-4 recruitment and activation. Activated TLR-4 results in increased inflammation causing, elevated GI stress and thus sets an ideal micro-environment for tumor development. Using a p47Phox, a functional sub unit of the NADPH oxidase system genetic knockout mice fed on a 60% kcal high fat diet that causes significant steatosis and insulin resistance, we show that TLR-4 activation and lipid raft recruitment is dependent on NADPH oxidase system. Mice fed on a normal chow diet were used as lean controls. Mice with a fatty liver showed higher expression of inflammatory markers such as IFN-γ, IL-1β, MCP-1, TNF-α in their GI tract whereas these effects were decreased in the lean control and the p47Phox knockout models. TLR 4 activation was also confirmed *in vitro* using Intestinal epithelial cells (IEC-6). The cells were treated with metabolites resulting from fatty acid metabolism in the liver such as 4-HNE, HMGB1, establishing a novel role of ectopic effects of liver metabolism on the GI tract. Thus for the first time we establish the role of a fatty liver and TLR-4 activation dependent on oxidative stress in creating a premalignancy phenotype in steatotic mice.

1853 Effects of Ipomoea Carnea in Placental Tissue. Evaluation in Rodents and Ruminants

A. T. Gotardo[2], L. L. Lippi[2], K. B. Violin[1] and S. L. Górniak[2]. [1]*Material Science and Technology Center, IPEN-University of São Paulo, São Paulo, Brazil and* [2]*Research Center of Veterinary Toxicology (CEPTOX), Department of Pathology, College of Veterinary São Paulo University, Pirassununga, Brazil.* Sponsor: K. Welsh.

Ipomoea carnea (IC) is a plant that is widely distributed in northeastern Brazil and in other tropical countries. Intoxication of livestock that chronically ingest IC has been reported in several countries, being goats the main affected species. IC contains the indolizidine alkaloid, swainsonine, as well as toxic calystegines. Swainsonine cause cellular accumulation of oligosaccharides, due to inhibition of several important enzymes,

resulting in cellular vacuolization and cell death in different organs. It is well known that IC ingestion during pregnancy leads to changes in fetal development in rats and goats; however, little is known about the effects of the IC in placenta. The aim of the present study was evaluate the effects of IC in the placental tissue of rats and goats. Pregnant rats of experimental group were treated orally by gavage, from gestation day (GD) 6 to GD19, with 7,0g/kg/day of IC aqueous fraction. Control group received only tap water by gavage. On GD20 cesarean section was performed and placental tissue was collected for histophatological and lectin-histochemistry (LHT) evaluation. Pregnant goats of experimental group were treated which IC fresh leaves at dose of 5g/kg/day, since GD35 until parturition. Control animals received no experimental treatment. Placental tissue was collected at parturition for histophatological and LHT evaluation. Placental tissue from experimental rats showed labirinth zone thickening and reduction of the junctional zone thickness, LHT revealed accumulation of sugars in some cells located at several regions of the placenta. Placental tissue from experimental goats showed cytoplasm vacuolization in columnar epithelium of chorioallantoic membrane, LHT revealed higher markup for the SWGA and WGA lectins suggesting that the vacuolated cells contain b-(1-4)-N-acetyl-glucosamine, and N-acetyl-neuramic acid in vacuoles, one of the main carbohydrates accumulated in this toxicosis. The results clearly revealed that the placental tissue is also target of toxic action of the toxic active ingredients present in the IC. Probably, changes in fetal development observed in these two animal species exposed to IC are also a consequence of the injury in the placental tissue that inevitably causes losses to the developing fetus.

1854 Air Pollution Induced Placental Epigenetic Alterations in Early Life: A Candidate miRNA Approach

K. Vrijens[3], M. Tsamou[3], N. Madhloum[3], W. Lefebvre[2], C. Vanpoucke[1], W. Gyselaers[3,5] and T. S. Nawrot[3,4]. *[1]Belgian Interregional Environment Agency, Brussels, Belgium; [2]Flemish Institute for Technological Research, Mol, Belgium; [3]Hasselt University, Diepenbeek, Belgium; [4]Leuven University, Leuven, Belgium and [5]Department of Obstetrics and Gynecology, East Limburg Hospital, Genk, Belgium.* Sponsor: H. Roels.

Background: Particulate matter (PM) exposure during *in utero* life may entail adverse health outcomes later in life. Epidemiological studies in adults have linked air pollution's adverse effects to alterations in gene expression profiles, which can be regulated by epigenetic mechanisms including microRNAs (miRNAs). Objectives: We investigate the potential influence of air pollution exposure in early life on placental miRNA expression. Methods: Within the framework of the ENVIRONAGE birth cohort, we measured the expression of miR-16, miR-20a, miR-21, miR-34, miR-146a and miR-222 by qRT-PCR in placental tissue from 211 mother-newborn pairs. Multiple regression models were used to study miRNA expression and *in utero* exposure to particulate matter over various time windows during pregnancy. *In silico* analysis was performed to predict genes and pathways targeted by the studied miRNAs. Results: Four out of six measured miRNAs were associated with air pollution exposure in early life. For each 5 µ/m3 increase in PM2.5 exposure during the 2nd trimester of pregnancy, the expression of miR-20a, miR-21, miR-146a and miR-222 at birth was reduced by 26.2% (95% CI:-45.2, -0.1, p=0.051), 33.8% (95% CI: -53.2, -6.4, p=0.021), 31.1% (95% CI: -48.1, -8.6, p=0.011) and 25.2% (95% CI: -47.2, -2.2, p=0.035). miR16 and miR-34a were not significantly associated with either PM2.5 or NO2 exposure over the different time windows analyzed. Pathway analysis revealed immune responses as the core pathways targeted by the studied miRNAs that were significantly associated with *in utero* air pollution exposure. Conclusions: Environmental exposure to particulate air pollution in early life can modify the expression of miR-20a, miR-21, miR-146a and miR-222 in human placental tissue. These miRNAs might be relevant targets involved in PM-induced effects in fetal programming and could potentially influence health outcomes later in life.

1855 Prenatal Exposure to Di-(2-ethylhexyl) Phthalate Affects Ovarian Follicle Numbers and Sex Steroid Hormones in the F1, F2, and F3 Generations of Mice

S. Rattan, E. Brehm, S. Niermann and J. A. Flaws. *Comparative Biosciences, University of Illinois at Urbana Champaign, Champaign, IL.*

Di-(2-ethylhexyl) phthalate (DEHP) is a plasticizer in building products. Humans are exposed to DEHP daily, but the effects of prenatal exposure to DEHP on female reproduction are not well understood. Thus, we tested the hypothesis that prenatal exposure to DEHP impairs the ovary and hormone levels in the F1, F2, and F3 generations of female mice. Pregnant CD-1 mice were orally dosed with corn oil (vehicle control) or DEHP (20 and 200 µg/kg/day, 200, 500, and 750 mg/kg/day) daily from gestation day 10.5 until birth (7-14 dams/treatment). F1 females were mated with untreated males to obtain the F2 generation, and F2 females were mated with untreated males to produce the F3 generation. On postnatal days (PNDs) 8, 21, and 60, female pups from each litter were euthanized, their ovaries were used for histological evaluation of follicle numbers, and their sera were used to measure estradiol, testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels. Prenatal DEHP exposure (500 mg/kg/day) decreases follicle numbers in the F2 generation and it (20 µg/kg/day) decreases follicle numbers in the F3 generation (n=2-5 dams/treatment; $p \leq 0.05$), but not in the F1 generation (n=3-10 dams/treatment). Prenatal DEHP exposure (500 mg/kg/day) also increases estradiol levels in the F1 generation and it (200 mg/kg/day) decreases estradiol levels in the F2 generation (n=3-14 dams/treatment; $p \leq 0.05$), but not in the F3 generation (n=2-8 dams/treatment). Prenatal DEHP exposure (20 µg/kg/day) decreases testosterone levels in the F3 generation (n=2-8 dams/treatment; $p \leq 0.05$), but not in the F1 generation or F2 generation (n=2-14 dams/treatment). Prenatal DEHP exposure does not affect LH and FSH levels in the F1-F3 generations. These data suggest prenatal DEHP exposure alters ovarian follicle numbers and sex steroid hormone levels in multiple generations of mice. Supported by NIH P01 ES022848, EPA RD-83459301, and T32 ES007326.

1856 Low Dose Tetrabromobisphenol A (TBBPA) Alters Mammary and Uterine Development Following Prenatal Exposure in Wistar Rats

S. E. A. Gillera[3], A. J. Filgo[3], D. K. Tucker[3], J. A. Flaws[2], B. Horman[1], H. B. Patisaul[1] and S. E. Fenton[3]. *[1]Biology, NC State University, Raleigh, NC; [2]Comparative Biosciences, University of Illinois, Urbana, IL and [3]Division of the of the National Toxicology Program, National Toxicology Program Laboratory, Research Triangle Park, NC.*

Tetrabromobisphenol A (TBBPA) is the highest production volume brominated flame retardant in the world. Due to widespread human exposure and little knowledge of its effects, TBBPA was evaluated in the National Toxicology Program 2-year bioassay, which indicated that adult TBBPA exposure contributed to uterine cancer development in rats. In this study, we evaluated a low dose, developmental oral exposure to TBBPA for endocrine disrupting activity in reproductive tissues. Wistar rats were orally exposed to 0 or 0.1 mg TBBPA/kg body weight from gestation day 8 to postnatal day (PND) 21. Mammary glands (male and female) and female reproductive tracts were obtained from offspring (N=5-8 litters represented/group) on PND 21 and at approximately 7 months of age. All females at 7 months were necropsied in the estrus stage of the cycle. Mammary gland whole mounts and histologic sections of mammary and uterine tissues were prepared. Blind evaluations were made by two reviewers for all tissues. Mammary gland development in male and female weanlings was delayed significantly by TBBPA exposure. Growth detriments included stunted epithelial outgrowth, decreased ductal budding, delayed terminal end bud development and prominent gland asymmetry compared to control glands. The epithelial abundance and growth delays were also noted in histologic evaluation of mammary sections at 7 months of age. There were also several significant differences between control and TBBPA-exposed uteri at 7 months. TBBPA exposure increased the number and size of endometrial glands, invagination of the endometrium, the luminal columnar epithelial cell height (2-3 fold), and caused an apparent increase in endometrial thickness and stromal density. There was also the appearance of highly mitotic bodies in the epithelial cells lining the lumen. These findings suggest that low doses of TBBPA act as endocrine disruptors to the male and female mammary glands and cause aberrant uterine development, with some effects suggestive of later life disease susceptibility.

1857 Enhanced Spontaneous Mammary Tumor Susceptibility Induced by BPA Alternatives

D. K. Tucker[2], S. A. Bouknight[1] and S. E. Fenton[3]. [1]*Charles River Laboratories, Inc., Durham, NC;* [2]*Curriculum of Toxicology, UNC at Chapel Hill, Chapel Hill, NC and* [3]*NTP Laboratory Branch, NIEHS, Research Triangle Park, NC.*

Early life exposure to human relevant doses of Bisphenol A (BPA) in several rodent models have resulted in altered mammary development with an increased susceptibility to tumorigenesis. Despite the manufacturer's willingness to remove BPA from consumer products, identifying its replacement chemicals including BPAF and BPS has lacked transparency. Both chemicals possess estrogenic qualities that may pose risks to the mammary gland during development and consequently long term effects which could include tumor formation. This study aimed to determine whether a perinatal exposure to BPAF and BPS would enhance the susceptibility to mammary carcinogenesis. Timed pregnant CD-1 mice were administered a twice daily oral gavage of vehicle, BPA (0.5, 5, or 50 mg/kg), BPAF or BPS (0.05, 0.5, or 5 mg/kg) between gestational days 10-17. Female offspring were sacrificed at 3, 8 and 14 months to obtain mammary glands for carmine stained whole mounts and histopathologic evaluation. Stromal hyperplasia was evident in both mammary whole mount and H&E sections of BPS 0.5 mg treated animals as early as 3 mo. Highly proliferative terminal end bud structures, which are commonly present during puberty, were still present in many glands at 8 mo. At this time approximately 60% (3/5) of BPAF 5 mg and 50% (2/4) BPS 0.5 mg whole mounts contained both inflammatory and hyperplastic lesions. 14 mo. old mice from the same dose groups exhibited a single or combination of lesions that included ductal squamous metaplasia, tubuloalveolar or papillary hyperplasia and an adenocarcinoma. BPA treated animals at this time-point also experienced pre-neoplastic lesions but at a much lower incidence compared to BPAF and BPS. Together, this data revealed that BPAF, BPS and BPA increased the incidence of pre-neoplastic lesions judged by whole mount and histopathology. Further studies will be necessary to determine the timing and mechanisms by which enhanced spontaneous tumor incidence may be occurring.

1858 Prenatal Exposure to Bisphenol A and High Fat Diets Alters Lifecourse Metabolic Outcomes

E. H. Marchlewicz, B. N. Sanchez, K. E. Peterson and D. C. Dolinoy. *Biostatistics, University of Michigan, Ann Arbor, MI; Environmental Health Sciences, University of Michigan, Ann Arbor, MI; Environmental Health Sciences, Nutritional Sciences, University of Michigan, Ann Arbor, MI; Nutritional Sciences, University of Michigan, Ann Arbor, MI.*

Prenatal exposures can impact development of metabolic disease across the life course. In this study we examined the hypothesis that lifelong metabolic effects of perinatal bisphenol A (BPA) are exacerbated by a Western high fat diet (HFD) and ameliorated by a Mediterranean HFD. Dams (n=98) were randomized to 1 of 6 diets: control (C), Mediterranean (M), Western (W), or each diet with 50ug BPA/kg chow, CBPA, MBPA, and WBPA, respectively. Metabolic parameters were assessed from postnatal day 10 (PND10, n=118) to 10 mos (10M, n=133), with extensive metabolic phenotyping at 2, 4, and 8 mos. ANOVA with Tukey's post-hoc analysis compared pups' metabolic outcomes between diet groups; mixed effects models will be used to assess repeated measures over time. At PDN10, CBPA weighed less than C pups ($p=0.001$); the impact of BPA was negated by both HFDs. Pups exposed to M had higher weekly weights from 5-10 mos of age ($p < 0.001$) and higher PND10 serum leptin levels ($p = 0.003$) and greater hepatic triglycerides ($p = 0.002$), compared to C. No differences were observed with BPA. M females were less active ($p < 0.02$) than C females at 2 and 4 mos. Results were similar but weaker among males. All significant findings were during the light cycle, suggesting HFD impacts resting activity. Fasting serum insulin at 8 mos was greater among M females ($p=0.026$) and males ($p=0.047$) than C. Preliminary plasma lipidomics in 10M females found lower circulating long-chain PUFA ($p < 0.05$) in perinatally exposed W compared to C, consistent with literature on biopsy-confirmed NAFLD. Additional lipidomics analyses are underway, expanding to male offspring and BPA exposed groups. Thus, perinatal HFD appears to have a greater impact on life course metabolic health than perinatal BPA exposure. Magnitude of some effects was sexually dimorphic supporting the need to conduct longitudinal studies in both male and female offspring. Lastly, lipid composition of the HFD matters, resulting in different longitudinal metabolic health outcomes.

1859 Prenatal Exposure to Bisphenol A and Hyperactivity: Implications for Attention Deficit Hyperactivity Disorder, a Systematic Review and Meta-analysis

J. R. Rochester, A. L. Bolden and C. F. Kwiatkowski. *The Endocrine Disruption Exchange (TEDX), Paonia, CO.* Sponsor: K. Howdeshell.

Background: Attention-deficit hyperactivity disorder (ADHD) has increased in prevalence in the past decade. Studies attempting to identify a genetic component have not shown strong associations, indicating there may be gene-environment interactions underlying the disorder, including early exposure to environmental chemicals. Methods: Using the Office of Health Assessment and Translation (OHAT) framework, a systematic review (SR) and meta-analysis (MA) was performed to determine the relationship between early life exposure to bisphenol A (BPA) and hyperactivity, a key diagnostic criterion of ADHD. Results: A review of the literature identified 25 rodent and 2 human studies. Studies included in the SR were conducted in gestational or prepubertal mice, rats, or humans via *in utero* or neonatal exposure. A random effects MA showed significantly increased hyperactivity in male rodents with early BPA exposure. In humans, gestational and postnatal BPA exposure appeared to be linked to hyperactivity in girls but not boys. Conclusions: These findings suggest there may be differential susceptibilities between sexes and species, possibly with respect to exposure period. We concluded that early life BPA exposure is a suspected human hazard for the development of hyperactivity. These results suggest that prenatal medical recommendations should include avoiding BPA exposure. SRs that result in hazard-based conclusions, such as the present analysis, should be used during the development of strategies to avoid environmental chemicals associated with neurodevelopmental health effects.

1860 Acute Exposure of Triclosan Induced Changes in Phase I and Phase II Enzyme Gene Expressions in Zebrafish

J. Yang, Z. Zhou and K.-M. Chan. *School of Life Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong.*

Triclosan (TCS) is a broad spectrum antibacterial agent. Previous studies suggested it to be safe, however, considering its rapid degradation to by-products, including methyltriclosan and dioxin, which may elicit more toxic effects to the ecosystem and human beings, TCS was removed from the EU list of provisional. In present study, both zebrafish (*Danio rerio*) and zebrafish liver cell line, ZFL, are employed to investigate the mechanisms of TCS-mediated toxicity and endocrine disruption. The 50% lethal concentrations (LC50) of TCS in zebrafish embryos were determined as 9.26 μM [24 h post fertilization (hpf)], 4.08 μM (48 hpf) and 1.20 μM (72 hpf and 96 hpf), respectively. Major deformity was the delayed hatching by 86.5% from 1.13 μM (327 ppb). Upon exposure on larvae, the LC50 values were comparable and ranging from 1.26-1.46 μM throughout 96 h post hatching (hph). Toxicity in ZFL cells were comparable between 24 h and 96 h time exposure, approximately at 9.0 μM. Ethoxyresorufin-o-deethylase (EROD) assay and Cyp1a level were applied *in vitro* to evaluate TCS biotransformation. Individual TCS exhibited no induction on Cyp1a activity, except a slight 2.6-fold induction at 5.0 μM. However, it significantly inhibited benzo-a-pyrene (BaP) (1 μM) induced Cyp1a activity from 2.5 μM. In 96 h treated mRNA expression profiling, cyp1a was not affected but the levels of cyp1c1, cyp1c2 and cyp2y3 were significantly inhibited. Genes encoding deiodinases (Dio 1/2/3), thyroid hormone receptor (TRβ) and transthyretin (TTR) were also studied to investigate TCS's thyroid hormone disruption potential. It was found that dio 2 and trβ were inhibited at 2.5 μM. More other drug metabolizing genes (e.g. Cyp3a) and hormone receptors are being investigated. On-going study includes mRNA profiling in 24 h short term exposed ZFL cells considering the short kinetics half life of TCS to be 10.8 h from human being based study. Moreover, three different luciferase base reporter gene systems, including TRβ, aryl hydrocarbon receptor (AhR) dependent xenobiotic response element (XRE) and pregnane X receptor (PXR), are employed to seek the interaction between the TCS and the potential nuclear receptors. (Supported by a Scheme II research funding from the Faculty of Science, CUHK)

1861 Acute Exposure of Lead Induced Changes in Hepatic Metabolizing Enzyme Gene Expressions in Zebrafish

J. Yang, C.-L. Leung and K.-M. Chan. *School of Life Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong.*

In many developing countries such as China, inappropriate handling of electronic wastes (e-wastes) has caused large scale chemical contamination leading to health concerns. Trace metals, mainly lead (Pb) and persistent organic compounds, mainly polybrominated diphenyl ether (PBDE), are the major pollutants., In present study, both zebrafish (Danio rerio) and a zebrafish liver cell line, ZFL, are employed to investigate the mechanisms of lead-mediated toxicity and endocrine disruption. The 50% lethal concentrations (LC50) of lead acetate in zebrafish embryos were determined at 24, 48, 72, 96 and 120 h post fertilization (hpf), respectively, ranging from 260 μM (85 ppm) to 129 μM (42 ppm). Major deformity was the delayed hatching by up to 45.2 % at 100 μM (33 ppm) at 120 hpf. Upon exposure on larvae, the LC50 were decreased remarkably from 250 μM (82 ppm) at 48 h post hatching (hph) to 13 μM (4 ppm) at 96 hph, indicating the lead accumulation in larvae blood and tissue because of its half life reported in these compartments as long as 40 days. Acute exposure to 10%, 25%, 50% of 96 h LC50 (1.15 mM or 374 ppm) *in vitro* in ZFL cells were conducted for both 24 h and 96 h, to study gene expression differentiations involved in hepatic metabolizing enzymes and thyroid hormone (TH) system. It was found that, phase I enzyme transcripts (cyp1a, cyp1b1, cyp1c1), phase II UDP-glucuronosyltransferase (ugt1ab) and the copper transporter proteins (atp7a, atp7b) were all significantly up regulated at 24 h, but no induction at 96 h. In contrast, all deiodinases (dio1/2/3) were inhibited to different extents, and TH receptor β (trβ) was induced at 96 h. The great changes of genes at short term exposure indicated the direct lead poisoning on liver and TH system. Ethoxyresorufin-o-deethylase (EROD) assay was further applied *in vitro*. Lead exhibited no induction on CYP1A activity upon individual exposure. However, antagonistic effect on benzo-a-pyrene (BaP) (1 μM) induced Cyp1a activity was observed from 160 μM (52 ppm). More other drug metabolizing genes (e.g. cyp3a) and hormone receptors are being investigated. On-going study recruits three different luciferase base reporter gene systems, including TRβ, aryl hydrocarbon receptor (AhR) dependent xenobiotic response element (XRE) and pregnane X receptor (PXR), to seek the interaction between the lead and the potential nuclear receptors.

1862 Weight-of-Evidence (WoE) Assessment of the Potential Effects of Perfluorohexanoic Acid on Endocrine Systems

R. Jung[1], N. Wang[2], I. Gaou[2], W. Buxton[4], S. Seiji[3] and A. Lynch[5]. *[1]Archroma, Reinach, Switzerland; [2]Arkema, Paris, France; [3]Asahi Glass Company, Ltd, Osaka, Japan; [4]Chemours Company, Wilmington, DE and [5]FluoroCouncil, Washington, DC.* Sponsor: A. Lynch.

Perfluorohexanoic Acid (PFHxA) is a potential degradation product of 6:2 Fluorotelomer compounds used to manufacture fluorotelomer-based products. PFHxA has been extensively studied for human safety and ecotoxicity. Current peer-reviewed and published *in vitro* and *in vivo* ecological and toxicological studies, including regulatory guideline toxicology studies conducted by FluoroCouncil member companies, up to April 2015 were assessed in a WoE approach based on the World Health Organization (WHO) 2002 definition of an endocrine disruptor for potential effects of PFHxA on endocrine systems. The WHO definition states, "an endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations." (WHO/IPCS, 2002). The data evaluated demonstrate that PFHxA is not a selective reproductive or thyroid toxicant in any species tested, is non-carcinogenic in a two year bioassay in rats, and is only moderately toxic in various repeated dose studies (Klaunig , 2015; Loveless, 2009; Chengelis, 2009; Iwai, 2014). The studies with No Observed Effects Levels (NOELs) determined for PFHxA in repeated-dose studies using mammals, birds or fish indicate no specific concern for the endocrine systems nor that endocrine effects determine the threshold for toxicity (Chengelis, 2009; Benningoff, 2011; Vongphachan, 2011; Cassone, 2012). Based on this WoE assessment of the current scientific literature and regulatory guideline toxicology studies, PFHxA did not produce an adverse effect on endocrine systems and therefore, cannot be considered an endocrine disruptor according to the definition of the WHO.

1863 Estrogen Receptor Beta Mediates Hepatotoxicity Induced by Perfluorooctane Sulfonates (PFOS) in Mouse Liver

C. Xu[2], Q. Liu[2], Z.-Y. Jiang[1] and A. Gu[2]. *[1]Department of Surgery, Ruijin Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, China and [2]Institute of Toxicology, Nanjing Medical University, Nanjing, China.* Sponsor: A. Gu.

Perfluorooctane sulfonate (PFOS), an artificial fluorosurfactant and global contaminant, was widely used in various consumer products. In this study, we aim to investigate the role of estrogen receptor β (ERβ) in PFOS-induced bile acids and cholesterol metabolism disorders using ERβ knockout mice exposed with PFOS gavage. Our results showed that 8 mg/kg daily dose of PFOS intake significantly induced hydropic degeneration and vacuolation in the hepatic cells, reduced bile acids and cholesterol levels in liver tissue, and influenced the abundance and composition of gut microbiota. It is important to note that, ERβ deficiency not only remarkably inhibited the morphological alteration of hepatocytes impairment, but also relieved disorders in bile acids and cholesterol metabolism caused by PFOS. Furthermore, the alteration of gut microbiota by PFOS intake was also modulated. The relative transcript abundance of key genes involving bile acids and cholesterol metabolisms exhibited similar changes. In conclusion, our investigation provided new evidences that ERβ mediated PFOS-induced hepatotoxicity and increased our understanding of the potential adverse effects of PFOS exposure on humans.

1864 Weight of Evidence Evaluation of Potential Endocrine Disruption by 1,3-Diphenylguanidine Based on Results of Tier 1 Endocrine Disruption Screening Program Assays

M. L. Kreider and J. M. Panko. *Cardno ChemRisk, Pittsburgh, PA.*

Under the Endocrine Disruption Screening Program (EDSP), the US Environmental Protection Agency (EPA) has established a set of assay protocols by which chemicals are to be initially screened for prioritization for endocrine disruption testing. These assays include five *in vitro* and six *in vivo* assays, covering estrogen, androgen, thyroid and steroidogenesis modes of action for endocrine disruption. The US EPA has advised that the results of these assays be evaluated using a weight of evidence approach to investigate consistency with respect to a proposed mode of action across and within assays. In this study, we tested the effects of 1,3-diphenylguanidine (DPG), an accelerator used in tire rubber vulcanization, in all 11 EDSP Tier 1 assays to determine potential to act as an endocrine disruptor. The results of these assays were then evaluated using a weight of evidence framework established by Borgert, et al. (2014). In this framework, individual assay endpoints are ranked according to their strength of association with and specificity for a mode of action hypothesis for endocrine disruption (i.e. estrogen agonist, estrogen antagonist, androgen agonist, androgen antagonist, thyroid agonist, thyroid antagonist, steroidogenesis promoter, steroidogenesis inhibitor). Greater weight was assigned to the endpoints with higher strength of association and specificity for a specific endpoint and this weighting was used to determine the overall weight of evidence for a chemical's potential to initiate endocrine disruption via the specified mode of action. DPG elicited few effects across all 11 assays. In the *in vitro* assays, DPG was only positive in the Estrogen Receptor Transcriptional Activation assay, and potency was relatively low compared to estradiol. In the *in vivo* assays, DPG produced sporadic effects across many assays, but these effects were deemed to be inconsistent with any given mode of action hypothesis. More importantly, none of the observed effects occurred in the Rank 1 endpoints, considered to be most closely associated with the mode of action hypotheses. Based on the weight of evidence analysis conducted for each of the eight endocrine disruption hypotheses, DPG is unlikely to act as an endocrine disruptor through any of the identified modes of action.

1865 Development of a Robust QSAR Model for Prediction of Estrogen Receptor Binding Using Large Data Sets

H. W. Ng[1], H. Ye[1], H. Luo[1,2], S. D. Sakkiah[1], W. Ge[1], W. Tong[1] and H. Hong[1]. [1]*NCTR/US FDA, Jefferson, AR and* [2]*University of Arkansas at Little Rock/University of Arkansas for Medical Sciences Bioinformatics, Little Rock, AR.*

Many chemicals have been found to interact with the endocrine system in the human body. The estrogen receptor α (ERα), which is amongst the most studied receptors, plays very important roles in the ER-mediated endocrine disrupting activities. With the aim of predicting estrogenic activity of chemicals to facilitate the evaluation of their endocrine interfering potential, we developed a classification model using an in-house pattern recognition algorithm Decision Forest (DF) to predict binding potential to ERα of chemicals. The DF model was developed using a large training data set of 3308 chemicals obtained from the US Food and Drug Administration's Estrogenic Activity Database (EADB) and evaluated using five-fold cross validations and challenged using the large external validation data sets of 1641 chemicals tested by 13 ER pathways specific high throughput screening *in vitro* assays obtained from the US Environment Protection Agency's ToxCast project. The DF model yielded an accuracy of 92% in the cross validations and an accuracy ranging between 70-80% in the external validations. Informative descriptor analysis was conducted which revealed 18 molecular descriptors that contributed significantly to more than 90% of the models in the cross validations and were considered to be the important features that contribute to the prediction ability of the DF model. Prediction confidence analysis revealed that the model had both high prediction confidence and accuracy for most chemicals. In summary, the results demonstrated that the model accurately predicted ER binding of chemicals and could be useful for assessing the ER-medicated endocrine activity potential of environmental chemicals.

1866 Is There an Added Value to the Incorporation of a Metabolizing System in Biodetection Assays for Endocrine Active Substances?

J. Mollergues[2], B. van Vugt-Lussenburg[1], M. Marin-Kuan[2], B. Schilter[2] and K. C. Fussell[2]. [1]*BioDetection Systems, Amsterdam, Netherlands and* [2]*Nestle Research Center, Nestec Inc., Lausanne 26, Switzerland.*

The use of *in vitro* assays is important for the biodetection of endocrine active substances (EAS); however such assessments fail to take into account the role of biotransformation on the activity of the tested substances. A method incorporating an S9 metabolic system into the CALUX-reporter gene assays for estrogen receptor α and androgen receptor -mediated activities has been developed. Methoxychlor (MC), which is known to exhibit increased estrogenic and anti-androgenic activities after biotransformation, was used as a proof of principle. The S9-application was optimized according to the source (animal species, inducers), concentration and treatment duration. As expected an increased estrogenic potency of MC was observed in presence of S9. For the anti-androgenic assay, a competing agonist not metabolized by S9 had first to be identified. However, biotransformation did not alter MC anti-androgenic activity. The method was then applied to test of benzo(a)pyrene and Bisphenol A estrogenicity, as well as flutamide anti-androgenicity. In these tests, metabolism was reflected in 1) shifts in potency, 2) increased receptor-mediated transcriptional activation or 3) both. These results demonstrate the relevance and feasibility of including biotransformation in *in vitro* bioassays for the detection of EAS.

1867 Developing an *In Vitro* Assay to Measure Key Signaling Events in Estrogen-Initiated Pathways and Guide Risk Assessment

M. M. Miller, D. L. Doheny, P. Balbuena, S. M. Ross, C. Deisenroth and R. A. Clewell. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

We use Ishikawa (IKA) cells as an *in vitro* model for examining dose-response for uterine effects of potential endocrine disrupting chemicals (EDCs). Estrogen signaling in the uterus is multifactorial, involving a network of response pathways that ultimately lead to proliferation, changes in cell survival, transcriptional responses, and altered cell-cell interactions. The proliferation assay used by US EPA ToxCast is based in a breast-cell and is sensitive to alterations in cell morphology. Due to demonstrated tissue-specific effects in human breast and uterus response, a uterine model is needed to predict safe human exposures. We identified an appropriate uterine cell (IKA) and confirmed that the cell model recapitulates protein, gene and proliferative responses to estrogens. Further, we compared the concentration response for these cells with human *in vivo* data to native ligand (estradiol), and the human drugs tamoxifen and ethinyl estradiol. The IKA proliferation model was highly sensitive and responded to these compounds at similar doses to normal adult women. To support screening activities, we have scaled this assay up to a higher throughput format. We have performed preliminary validation studies with a set of EDSP21 compounds to compare the accuracy of our methods to the assays now in the ToxCast repertoire and will be expanding to a larger set of compounds. Preliminary results indicate that this assay has higher sensitivity, in terms of activating dose, than current HTS assays. We have also successfully multiplexed the proliferation assay with viability markers and a readout for estrogen receptor mediated transcription. Our goal in this project is development of a validated fit-for-purpose functional assay which is representative of *in vivo* uterine biology and capable of predicting points of departure for an *in vitro*-based chemical safety assessment.

1868 Effect of Bisphenol A on the Fetal Production of Pituitary and Testicular Hormones and Its Mechanism

T. Takeda[2], Y. Kariyazono[2], M. Hitomi[2], Y. Hattori[2,3], S. Narimatsu[1], Y. Ishii[2] and H. Yamada[2]. [1]*Graduate School of Medicine, Densitory and Pharmaceutical Sciences, Okayama University, Okayama, Japan;* [2]*Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan and* [3]*Research Fellowship for Doctoral Course Students, Japan Society for the Promotion and Science, Tokyo, Japan.*

Bisphenol A (BPA), a material used for the industrial production of polycarbonate and epoxy resins, has been suspected to have harmful effects on living bodies through the endocrine disruption. Previous reports have suggested that maternal exposure to BPA at lower doses produces a number of developmental disorders including sexual immaturity and neural abnormality in the offspring. However, the mechanism underlying these disorders remains poorly understood. To address this issue, we investigated here whether BPA affects the pituitary-gonadal axis in fetuses to alter steroidogenesis. Single oral administration of BPA (40 μg/kg) to pregnant rats at gestational day (GD) 15 reduced the expression of steroidogenic acute-regulatory protein (StAR), a rate-determining protein for steroidogenesis, in the testes of fetuses at GD20. The expression of pituitary luteinizing hormone (LH), an upstream regulator for testicular steroidogenesis, and its receptor in the testis was insensitive to the treatment. Neither a pathological change in the testis nor the status of the CpG methylation of StAR gene was observed in BPA-treated fetuses. However, in the cultured fetal testis, BPA suppressed the LH-stimulated expression of StAR mRNA. These results demonstrate that BPA directly targets an LH-dependent signaling to attenuate steroidogenesis in the fetal testis. In addition, maternal exposure to BPA attenuated the pituitary expression of growth hormone in a dose-dependent fashion. From an analysis using flow cytometry, such reduction is thought to be due to BPA-promoted apoptosis in the fetal pituitary. These results suggest that gestational exposure to BPA decreases the fetal production of several hormones in the pituitary and testis through multiple mechanisms, resulting in fetal undergrowth.

1869 Testing the Waters to Account for Metabolism in the T47D-kbluc Estrogen Receptor Transcriptional Activation Assay

B. R. Hannas, L. Kan, K. Johnson, L. Luna, M. LeBaron and R. Rasoulpour. *The Dow Chemical Company, Midland, MI.*

With the advent of Toxicity Testing in the 21st Century, high-throughput *in vitro* screening assays have gained increasing momentum in the area of predictive toxicology. Despite these advances, a critical challenge faced in moving towards *in vitro*-based assays for compound screening and safety testing is accounting for chemical metabolism. There is a vast body of literature describing compounds that are activated or inactivated for endocrine disrupting activity through *in vivo* metabolism. Therefore, screening of a compound in a metabolically incompetent *in vitro* assay does not provide a complete representation its potential endocrine activity. We tested the hypothesis that incubating a compound with primary rat hepatocytes and subsequently transferring the metabolite-containing hepatocyte media into an estrogen receptor transcriptional activation (ER-TA) reporter assay (T47D-kbluc) will recapitulate metabolism-dependent *in vivo* ER activity. A series of assessments were performed using the T47D-kbluc ER-TA to determine cell viability, ER-responsiveness and background estrogenic potential in the presence of hepatocyte media. Several positive controls consisting of known proestrogen compounds (methoxychlor (MXC), chalone, trans-4-Phenyl-3-buten-2-one (t-PBO), diphenylpropane (DPP),

trans-stilbene, trans-stilbene oxide) and estrogenic compounds known to be inactivated through metabolism (benzyl butyl phthalate (BBP), bisphenol S (BPS)) were assessed in this format. Results from the ER-TA assay demonstrated that estrogenic activity of the test compounds was altered following incubation with hepatocytes, suggestive of metabolic activation or inactivation. Specifically, a shift in the dose response curve was observed for high concentrations of MXC following incubation with hepatocytes, suggesting conversion to the more potent metabolite, 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE). Conversely, estrogenic activity of BBP was completely ablated following incubation with hepatocytes, indicative of cleavage of the parent compound to the non-estrogenic metabolites. Efforts are ongoing to analytically identify metabolites in both hepatocyte-treated and ER-TA assay media. This approach provides a solid starting point to account for metabolism in *in vitro* toxicology and consider the impact of metabolism on endocrine activity in an ER responsive *in vitro* screening assay.

PS 1870 Enhancement of the Endocrine Disruptor Knowledge Base for Assessing Endocrine Activity of Untested Chemicals

H. Hong, H. W. Ng, S. D. Sakkiah, W. Ge, H. Ye, H. Luo, G. Zhou, H. Fang, R. Perkins and W. Tong. *NCTR, US FDA, Jefferson, AR.*

Endocrine disruptors (EDs) are exogenous chemicals with potential to disrupt the endocrine system and came under international scientific scrutiny two decades ago. Regulatory attention followed that led FDA to develop the Endocrine Disruptor Knowledge Base (EDKB) in 1996. Since then, the publically available EDKB has been widely used by the scientific community. Recently, we updated the estrogenic data and augmented the EDKB with a comprehensive database of chemicals assayed for estrogenic activity, EADB. The database now contains over 21,000 estrogenic activity data points across more than 8,000 compounds assayed among 11 species. EADB also contains protein subtypes and domains used in the assays. Original data were converted into the same units, such as logRBA for competitive receptor binding data. Quality control was performed. In addition to EADB, data for binding affinity to rat α-fetoprotein (AFP) and human sex hormone binding globulin (SHBG) for 125 chemicals were included in EDKB. EDKB also incorporates models for predicting ER estrogenic activity for untested chemicals based on the expanded data. A model for predicting antagonist or agonist activity of ER binders based on competitive molecular docking was developed and included in EDKB. The model had an accuracy of 91.5% for agonist prediction and 89.5% for antagonist prediction. We demonstrated EDKB is a useful resource for assessment of endocrine activity of chemicals. EDKB will continually be improved to provide the scientific and regulatory communities with an open means to search endocrine activity data and develop predictive models for predicting endocrine activity.

PS 1871 Isolation, Culture and Characterization of Human Uterine Cells for Comparison to Current Estrogen Screening Assays

P. Balbuena, S. M. Ross, S. Rowley, M. Miller, B. Foley and R. A. Clewell. *Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Biologically relevant *in vitro* models are essential for characterizing toxicity pathways and moving beyond screening to predicting safe chemical exposures from human cell based assays. For estrogenic responses, there are many assays available for screening compounds, but very few have been developed in biologically relevant cell systems. Further, despite demonstrated differences in human uterine and breast response to xenoestrogens, current *in vitro* screening batteries (ToxCast™, Tox21) do not include a uterine assay. We are working to develop an *in vitro* uterine model (Ishikawa, IK) capable of capturing *in vivo* response to uterotrophic compounds, both in terms of dosimetry and phenotypic response (proliferation). To compare the IK model to normal human uterine cells, we developed an isolation and cell culture method for human endometrium epithelial primary cells (HEECs) collected from human tissue. Estrogen receptor expression and epithelial origin were verified by staining for epithelial markers (EpCAM, CK-18). The cells can be passaged at least three times without morphological or phenotypic changes. Phenotypic response to ethinyl estradiol (EE) was compared to several *in vitro* systems including IK and a breast epithelial carcinoma cell (MCF7). HEEC proliferation (1.8-fold change) was induced with >10 pM EE. IK and MCF7 cells showed less induction (1.2-fold), but MCF7s induced proliferation at lower doses than HEEC or IK cells. IKA and HEEC dose-response was similar, both increased proliferation 10 pM. These studies support the use of the IK cell line to predict dose-response in uterine cells. They also underscore the need for development of fit-for-purpose assays that account for cellular context and include key biological components of the toxicity pathway if we are to move forward to quantitative use of *in vitro* assays in safety assessments.

PS 1872 Investigating the Role of Bisphenol-A in Benign Prostatic Hyperplasia

J. Wynder[5], T. Nicholson[5], J. Vellky[5], T. Liu[5], C. Jeong[5], F. v. Saal[2], R. Wood[6] and W. Ricke[5]. *[1]Department of Urology, University of Wisconsin-Madison, Madison, WI; [2]Divison of Biological Sciences, University of Missouri-Columbia, Columbia, MO; [3]McArdle Cancer Biology Program, University of Wisconsin-Madison, Madison, WI; [4]Molecular and Environmental Toxicology, University of Wisconsin-Madison, Madison, WI; [5]School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI and [6]Urology, University of Rochester School of Medicine & Dentistry, Rochester, NY; [7]Urology, University of Wisconsin-Madison, Madison, WI.*

Benign prostatic hyperplasia (BPH) occurs in 80 percent of men over the age of 70 and is characterized by enlargement of the prostate, hyperplasia, and associated with lower urinary tract dysfunction. If left untreated BPH leads to enlarged bladder and ultimately urinary retention. Steroid hormones such as testosterone (T) and 17-B estradiol (E2) are known to play a role in the development of BPH but the impacts of environmental estrogens such bisphenol-A (BPA) are currently unknown. The aim of this study was to evaluate the effects of BPA on the induction of BPH in adult male mice and evaluate BPA regulated genes. Adult male mice were treated with slow release subcutaneous T+BPA, T+E2, or untreated for four months as we have described previously. Mice treated with T+BPA developed significantly ($P<0.05$) enlarged prostates and bladders compared to control untreated mice and were similar to T+E2 positive controls. Using gene expression microarray analysis, we identified integrin-B1(IGBB1), an extracellular matrix receptor associated with metastasis and migration, as a BPA down-regulated gene in prostate stromal cells grown in culture. QPCR analysis confirmed that BPA significantly reduced ITGB1 mRNA in T+BPA treated prostates. We also performed immunohistochemistry in mouse prostates and observed a significant reduction in ITGB1 localization in T+BPA treated mice. We conclude that BPA behaves similar to E2 to induce BPH and lower urinary tract dysfunction. These findings highlight the importance of investigating the impacts of environmental estrogen responsive genes and their association with BPH. Source of Funding R01 DK093690, U54 DK104310, U01 ES10-009, RC2ES018764, and T32ES007015(J.L.W)

PS 1873 Effect of Subchronic Treatment with Genistein on Testicular Cytochrome P450 (CYP) Enzyme Expression in Adult Male Rats

H. Chung[2], W. Vogl[2] and S. M. Bandiera[2]. *[1]Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada and [2]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada.*

Genistein, a phytoestrogen and isoflavone, produces many biological effects in mammalian cells, including inhibition of tyrosine kinase and topoisomerase II and antioxidant activity. Genistein binds weakly to estrogen receptors and can mimic estrogen in the body. In humans, the major route of exposure is through consumption of soy beans and soy-based food products. In the present study, 7-week old male Sprague-Dawley rats were randomized into five treatment groups with 6 rats per group. Rats received daily subcutaneous injections of genistein at 4, 40 or 400 µmol/kg/day, or estradiol benzoate at 0.4 µmol/kg/day, or vehicle at 1 ml/kg/day, for 2 weeks. Body weights were recorded daily and body weight gain from day 1 to day 15 was calculated. One day after the last treatment, rats were killed and their liver and testes were immediately excised and weighed. Testicular microsomes were prepared from individual rats and expression of CYP1B1, CYP2A1, CYP17A1, microsomal epoxide hydrolase (mEH) and CYP oxidoreductase (POR) was analyzed by immunoblots and immunohistochemistry. Body weight gain was similar for vehicle-treated rats and rats treated with genistein at all three dosages, whereas body weight gain of rats that were treated with estradiol benzoate was decreased by 38% compared to the vehicle-treated group. There was no difference in testis or liver weight regardless of the treatment group. Testicular protein levels of CYP2A1 and mEH were decreased by 72% and 54%, respectively, for rats treated with genistein at the highest dosage, and by 93% and 74%, respectively, for rats treated with estradiol benzoate compared with the vehicle-treated

group. Similarly, treatment with genistein or estradiol decreased testicular CYP17A1 and POR expression. Immunoblot analysis of CYP1B1 and immunohistochemical analysis of testis cryosections are currently in progress. The results to date indicate that genistein can suppress testicular CYP enzyme expression in adult male rats.

1874 Uterine Gene Expression and Response to 7,12-Dimethylbenz(a)anthracene (DMBA) Differ in Ovariectomized Rats Fed Soy Protein Isolate or Treated with 17 Beta Estradiol

M. J. Ronis[2], M. Blackburn[1], H. Gomez-Acevedo[1], K. Shankar[1], R. Singhal[1], M. A. Cleves[1] and T. M. Badger[1]. *[1]Arkansas Children's Nutrition Center, University of Arkansas for Medical Sciences, Little Rock, AR and [2]Pharmacology & Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, LA.*

There are concerns regarding possible reproductive toxicity from consumption of soy foods, including an increased risk of endometriosis and endometrial cancer, as a result of the presence of phytoestrogens. In the current study, female rats were fed AIN93G diets made with either casein (CAS) or soy protein isolate (SPI) from postnatal day (PND) 30, ovariectomized on PND 50 and infused with 5 µg/kg/d 17β-estradiol (E2) or vehicle. E2 increased uterine wet weight ($P<0.05$). RNAseq analysis revealed that E2 significantly altered expression of 1991 uterine genes ($P<0.05$), while SPI feeding had no effect on uterine weight and altered expression of far fewer genes than E2 at 152 genes ($P<0.05$). Overlap between E2 and SPI genes was limited to 67 genes. GO analysis indicated significant differences in uterine biological processes affected by E2 and SPI and little evidence for recruitment of estrogen receptor α to the promoters of ER responsive genes after SPI feeding. E2 up-regulated genes were prominent in uterine cancer signaling pathways and extracellular matrix organization; whereas. SPI feeding up-regulated uterine peroxisome proliferator activated receptor (PPAR) signaling and fatty acid metabolism. The combination of E2 and SPI resulted in significant regulation of 504 fewer genes relative to E2 alone. The ability of E2 to induce uterine proliferation in response to the carcinogen dimethybenz(a) anthracene (DMBA) as measured by expression of PCNA and Ki67 mRNAs ($P<0.05$) was blocked in SPI-fed rats. These data suggest SPI is a selective estrogen receptor modulator (SERM) interacting with a small sub-set of E2-regulated genes and is anti-estrogenic in the presence of endogenous estrogens. Supported in part by United States Department of Agriculture, Agricultural Service Current Information System Grant 6251-51999-007-04S.

1875 Characterization of Xenobiotic Defense Mechanisms of the Pancreatic Beta Cell

C. M. Smith[2], E. M. Bobczynski[2], V. R. Borges[2], T. M. Bull[2], E. Heart[1] and J. P. Gray[2]. *[1]Molecular Pharmacology & Physiology, U. South Florida, Tampa, FL and [2]Science, US Coast Guard Academy, New London, CT.*

Pancreatic islet beta cells contribute to glycemic control by secreting insulin in response to postprandial elevation in blood glucose levels. While INS-1 832/13, a rat insulinoma glucose-responsive beta cell line, is heavily utilized to study pancreatic beta cell metabolism and glucose-stimulated insulin secretion (GSIS), the usefulness of this cell line for toxicological studies has not been fully established. In the current work, we investigated the response of the INS-1 832/13 cells to the inducers of the cytochrome P450 pathway (carbamazepine, dexamethasone, rifampicin, and beta-naphthoflavone), the nrf2 inducer CDDO-Im, and the DNA replication inhibitor doxorubicin. Basal expression of cytochrome P450 genes was extremely low in these cells, in many cases 1000-fold less than the expression found in the rat liver when compared with the expression of beta-actin. Out of more ten other cytochrome P450 genes screened, only cytochrome P450 1A1 was induced by treatment with dexamethasone. However, CDDO-Im (100 nM) caused a 9-fold induction in the gene expression of nrf2 targets NQO1 and HO-1 and doxorubicin caused a dose-dependent increase in the gene expression of cell cycle and apoptotic genes including mdm2 and fas. Taken together, these data suggest that while the nrf2 and DNA damage pathways are active, the cytochrome P450 system is largely non-functional in these cells. Future studies will evaluate the responsiveness of intact islets of Langerhans to toxicants to determine the status of these protective systems *ex vivo*.

1876 Evaluation of Perfluorooctane Sulfonate (PFOS) and 2,2′,4,4′,5-Pentabromodiphenyl Ether (BDE-99) Stimulation of Insulin Secretion from INS-1 832/13 Pancreatic Beta Cells

E. Heart[2], J. P. Gray[3] and A. L. Slitt[1]. *[1]College of Pharmacy, U. of Rhode Island, Kingston, RI; [2]Molecular Pharmacology & Medicine, U. of South Florida, Tampa, FL and [3]Science, US Coast Guard Academy, New London, CT.*

Perfluorinated and polyfluorinated compounds (PFCs) and Polybrominated diphenyl ethers (PBDEs) are environmentally persistent chemicals, with increasing concern for metabolic health effects. PFCs are widely used stain- and grease-repellent chemicals and present in fire fighting foams. Increased PFC exposure in overweight 8- to 10-year-old children has been associated with higher insulin and triglyceride concentrations. PBDEs are utilized as flame retardants, with BDE-99 relatively abundant in commercial mixtures. Less evidence exists regarding an association between PBDE exposure and diabetes. In this work, we evaluated the effect of PFOS and BDE-99 on insulin secretion and overall cell health using the rat insulinoma beta cell line INS-1 832/13 to determine if enhanced insulin secretion could be a contributor to hyperinsulinemia and subsequent peripheral insulin resistance. Cells were treated either acutely (1 h) or chronically (48 h) with PFOS or BDE-99 (1-25 µM) and examined for insulin secretion at basal (3 mM) or stimulatory (16 mM) concentrations of glucose for 1 h. Acute exposure to PFOS caused a dose-dependent increase in insulin secretion at both basal and stimulatory concentrations of glucose. PFOS also increased the insulin secretion stimulatory index, measured as the ratio of secretion at stimulatory versus basal concentrations, from 3-fold to 4- and 5-fold upon addition of 1 and 5 µM PFOS, respectively. PFOS had no effect on cell growth and viability as measured by cell titer blue reduction at doses less than 400 µM for 48 h, suggesting that the increases in insulin secretion due to PFOS were not due to overt toxicity. Acute treatment with BDE had no impact on insulin secretion at the doses tested (1-25 µM). To evaluate the effect of chronic exposure to PFOS or BDE, cells were treated with these compounds for 48 h and the chemicals were removed prior to the testing of the cells for insulin secretion. While PFOS had no effect on insulin secretion, BDE caused a decrease in insulin secretion at both basal and stimulatory glucose, suggesting overt toxicity. Taken together, these data show that PFOS can stimulate insulin secretion and therefore beta cell dysfunction may contribute to the development of metabolic disorders induced by flame retardants.

1877 The Effect Of DDE Exposure on Free Fatty Acid Regulation of Liver Cell and Beta Cell Function and Its Association with Type 2 Diabetes

A. B. Ward, M. B. Dail and J. E. Chambers. *Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.*

Type 2 diabetes (T2D) is a metabolic disease characterized by insulin resistance, dyslipidemia, and beta cell dysfunction. Epidemiological evidence indicates an association of organochlorine compounds such as dichlorodiphenyldichloroethylene (DDE), the bioaccumulative metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), with T2D. Lipids including free fatty acids and their metabolites are often elevated in T2D and long-term DDE exposure has been associated with increased lipid levels in diabetics and non-diabetics. The purpose of this study was to investigate the *in vitro* effect of DDE exposure on free fatty acid induced lipid secretion from McArdle-RH7777 (McA) rodent liver cells and insulin secretion from Beta-Tumor Cell-6 (B-TC-6) murine pancreatic beta cells. Cells were exposed to DDE for 24 hours with or without co-exposure to oleic acid, a free fatty acid. Levels of markers of T2D including apolipoprotein B (ApoB), triglycerides (TG), and insulin were measured. DDE exposure decreased intracellular TG levels and increased ApoB secretion from liver cells. Protein levels of intracellular lipid transporters varied: microsomal triglyceride transfer protein increased while sortilin-1 decreased. DDE exposure increased insulin secretion from beta cells. Protein levels of the insulin transcription factor, pancreatic and duodenal homeobox factor-1, and enzyme activity of the insulin translation factor, prohormone convertase, increased. DDE exposure may exacerbate the effects of free fatty acid induced lipid secretion from liver cells and insulin secretion from beta cells. These *in vitro* data complement the *in vivo* association of DDE exposure with T2D by suggesting a mechanistic effect of DDE exposure.

1879 Effects of Zinc Status on Obesity-Induced Metabolic Disorder, and Cardiac Hypertrophy in Young Mice

S. Wang, M. Luo and L. Cai. *Kosair Children's Hospital Research Institute, University of Louisville, Louisville, KY*. Sponsor: L. Cai.

Using a murine model of childhood obesity, we investigated the effect of dietary zinc deficiency and supplementation on metabolic disorder and cardiac hypertrophy in mice consuming a high-fat diet (HFD). Mice at 4-weeks of age were divided into two groups and fed either the childhood obesity model diet (HFD, 60% kcal fat) or normal diet (ND, 10% kcal fat). These groups were further subdivided according to varied Zn supplementation for 3 or 6 months: low (ZD), normal (ZN), or high (ZS); 10, 30, 90 mg zinc per 4057 kcal, respectively. The mechanistic study was done in HFD-fed mice treated with and without p38MAPK inhibitor (SB203580). Results showed that HFD induced significant insulin resistance, hyperinsulinemia, hypertriglyceridemia, and cardiac hypertrophy. HFD also significantly increased B-cell CLL/lymphoma 10 (BCL10)/caspase recruitment domain (CARD9) expression, p38 MAPK, and Nuclear factor kappa-B activation. These obese-linked effects were worsened in HFD/ZD mice and attenuated in HFD/ZS mice. Immunoprecipitation results suggested increase BCL10/CARD9 interaction in the HFD group, which was further increased in the HFD/ZD group and attenuated in the HFD/ZS group. The cardiac pathological changes were significantly evidenced in a time-dependent manner from 3 months to 6 months. Furthermore, inhibition of p38 MAPK completely abolished HFD-induced, and even zinc deficiency-worsened, cardiac effects except for BCL10/CARD9 expression. These results suggest that HFD and zinc deficiency synergistically induce an obesity-related systemic lipid profile and cardiac hypertrophy via p38 MAPK activation mediated hypertrophy and BCL10/CARD9 interaction. Zinc supplementation prevented HFD-induced cardiac hypertrophy, suggesting the potential administration of zinc to prevent juvenile obesity linked cardiac hypertrophy.

1880 Toxicity Assessment of Sprague Dawley Offspring Exposed to 2-Aminoanthracene *In Utero*

R. Toure and W. E. Gato. *Chemistry, Georgia Southern University, Statesboro, GA.*

Environmental exposures to toxic chemicals increase the risk of developing diseases such as type 2 diabetes. 2-Aminoanthracene (2AA) is an aromatic amine commonly found in the environment including road tars, dyes, and cigarette smoke. Previous studies have found a link between 2AA and insulin-dependent gene expression changes. The goal of the present investigation is to examine the expression of a select pancreatic genes in response to 2AA *in utero*. Timed Sprague Dawley pregnant rats were fed 0 mg/kg (control), 50 mg/kg (low dose), and 100 mg/kg (high dose) concentrations of 2AA diet. After sacrifice, a histological examination of the pancreas was performed to determine the microscopic anatomy of pancreatic cells followed by specific insulin staining and β-cell mass analysis. Quantification of insulin-dependent transcripts; FTO, GCK, IGF2BP2, PPARG1, GLUT2 and INS1 via qRT-PCR is underway. Insulin IHC staining indicated that pancreatic islet of the low dose progeny had the highest total percentage of pancreatic tissue, followed by the control offspring and then the high dose exposed rats. This experiment is ongoing to examine how these parameters change with moderately high fat diet three months postwean. Also, clinical chemistry of pancreatic enzymes will be undertaken. Quantification of the expression of genes associated with the regulation of insulin and obesity might suggest a link between diabetes and exposure to environmental chemicals such as an aromatic amine.

1881 ToxCast Outcomes for Biphenyl Indicate Concordance with Recognized *In Vivo* Biological Effects

J. Klapacz[2], S. Green[1], D. M. Wilson[2] and A. Parks[2]. [1]*The Eastman Chemical Company, Kingsport, TN and* [2]*TERC, The Dow Chemical Company, Midland, MI.*

ToxCast is a program of the US EPA that utilizes *in vitro* cellular and biochemical assays to screen chemicals for their potential to disrupt biological pathways. ToxCast outputs can indicate a chemical's potential toxicity and *in vivo* mechanistic outcomes. Biphenyl (BP) is an aromatic hydrocarbon with an extensive toxicological dataset that has been used in closed-system applications. Recently, BP has been tested in 386 of the ToxCast high throughput screening (HTS) assays. The analysis of ToxCast outputs indicated that BP is active in seven of these assays; the two most active ones were NVS_IC_rKCaCh (calcium ion channel for neurotransmission) and BSK_CASM3C_MCP1_up (chemokine involved in immunoregulation), with the half maximum activity values (AC50s) of 0.00024 and 0.0154 μM, respectively. Other biological pathways activated in ToxCast by BP were maintenance of tissue homeostasis & proliferation, response to inflammation, and cellular signaling & cell growth. BP was also active in two estrogen (ER) assays, but was not active in thyroid or androgen assays. BP activated the RNA Transcription node of the ER pathway only, containing the ATG_ERa_TRANS_up and ATG_ERE_CIS_up assays with AC50s of 22.1 and 84.2 μM, respectively; these AC50s were >1000-fold less active compared with the two most active outcomes. There are 18 HTS ToxCast assays for the ER pathway and such sporadic activation together with low potency do not suggest BP would act via an endocrine (ED) mode-of-action (MOA). In support, the traditional toxicological database does not suggest ED activity for BP and implicated liver and kidney to be the primary targets for toxicity. Furthermore, BP is extensively metabolized *in vivo* and detoxified by conjugation with sulfate and/or glucuronide, or methylation. Taken together, the absence of any significant findings of ER activation in ToxCast along with the absence of ED effects *in vivo* indicate that ED MOA is not relevant for BP and that BP may primarily interact with other biological pathways. Lastly, the disruption of tissue hemostasis and activation of cell growth and signaling pathways are consistent with regenerative proliferation MOA for liver tumors reported in female mice.

1882 Validation of High-Throughput Screening Data and Novel Mechanistic Insights into VDR-Xenobiotic Interactions by Orthogonal Assays

D. Mahapatra[1], J. A. Franzosa[2], K. A. Houck[2] and S. W. Kullman[1]. [1]*Biological Sciences, NCSU, Raleigh, NC and* [2]*US EPA, Research Triangle Park, NC.*

Exposure to environmental neurotoxicants can lead to neurobehavioral disorders in humans. Vitamin D in recent years has emerged as crucial player driving embryonic neurodevelopment, neuroprotection from xenobiotics and its deficiency has been implicated as a risk factor for development of neurodegenerative diseases such as Parkinson's disease, Schizophrenia, and Multiple sclerosis. A quantitative high-throughput (qHTS) screen using the US EPA ToxCast Data Analysis Pipeline generated ~90 potential VDR agonists and ~380 potential VDR antagonists. We identified 26 agonists and 24 antagonists based on potency, efficacy, and VDR selectivity and validated them using our in-house luciferase reporter gene assays. Transient transactivation assay (TT) using a human VDR plasmid and Cyp241A1 luciferase reporter construct in Hek 293T cells revealed 22/26 active VDR agonists and 23/24 active VDR antagonists. Data was consistent when a zebrafish VDR alpha construct was additionally tested. Compounds were then examined using mammalian-2-hybrid assay (M2H) to evaluate VDR interactions with co-activators and co-regulators. With the exception of a select few compounds, VDR agonists exhibited moderate recruitment of co-regulators and co-activators whereas antagonists exhibited moderate to marked attenuation of co-activator recruitment by vdr both in the presence and absence of co-regulators. Overall, the data emphasizes the molecular complexity of VDR transcription machinery in terms of differential and preferential affinities of compounds for co-regulators/co-activators and respective sites of action. Further research will help decipher pathways that these chemicals follow to attain functional outcomes.

1883 Tributyltin Engages the RXR And LXR Nuclear Receptor Pathways to Modify Osteoblast/Osteoclast Crosstalk and Enhance Bone Deposition in a Sex-Dependent Manner

J. Watt[3], A. Baker[4], B. Meeks[4], E. Morgan[2], L. Gerstenfeld[4] and J. Schlezinger[3]. [1]*Boston University School of Medicine, Boston, MA;* [2]*Bioengineering, Boston University College of Arts and Sciences, Boston, MA;* [3]*Environmental Health, Boston University School of Public Health, Boston, MA and* [4]*Orthopaedic Surgery, Boston University School of Medicine, Boston, MA.*

The balance of bone building and resorption is maintained by osteoblasts and osteoclasts and is coordinated by activation of nuclear receptors (e.g. liver X receptor (LXR), peroxisome proliferator activated receptor γ (PPARγ), retinoid X receptor α (RXRα)) and transcription factors (e.g. Runx2, Nfatc1). Therapeutics and toxicants that interact with nuclear receptors can influence cell differentiation, cell function, and osteoblast/adipocyte-osteoclast communication, with consequences to bone microarchitecture. Tributyltin (TBT) is a dual PPARγ/RXRα agonist that activates RXR, PPAR and LXR pathways in bone marrow-derived multipotent mesenchymal stromal cells (MSC) and modifies MSC differentiation *in vitro*. Here, we tested the hypothesis that TBT is a negative regulator

of bone quality *in vivo*. Whereas previous studies have shown that TBT suppresses osteogenesis *in vitro* and PPARγ agonist (i.e. rosiglitazone) exposure decreases bone density *in vivo*, TBT (10 mg/kg, oral gavage, 3x per week) increased trabecular microarchitectural parameters (e.g. bone volume and trabecular thickness) while decreasing cortical thickness in female C57Bl/6J mice treated from 12-22 weeks of age, compared to those treated with vehicle. In contrast, TBT only reduced cortical thickness in male C57BL/6J mice. We hypothesize that TBT modifies the osteoblast/osteoclast balance in females via activation of LXR through permissive activation of RXRα. In a reporter assay, TBT directly activated RXRα, and upregulated RXR homodimer and RXR/LXR target genes in osteoblasts. Ongoing experiments are investigating if the female phenotype is driven by direct effects on osteoclasts (e.g. a suppressive effect of TBT on osteoclast differentiation or resorptive capacity, enhanced production of osteoblast-supportive IL6 family cytokines) or by direct effects on osteoblasts (e.g. suppression of RANKL production). These studies show that the environmental toxicant TBT modifies bone homeostasis and support a central role for RXRα in regulating bone health.

PS 1884 Neurodevelopmental Endpoints Modulated by Thyroid Hormone in Primary Rat Neuron-Glia Co-Cultures

K. A. Hayakawa, K. M. Walter and P. J. Lein. *Molecular Biosciences, University of California, Davis, Davis, CA.*

It is widely postulated that fetal and/or infant exposure to environmental chemicals that interfere with thyroid hormone (TH) signaling during critical periods of development may contribute to the increased incidence of neurodevelopmental disorders. This hypothesis is derived from clinical observations that insufficient levels of TH during gestation are associated with mild to severe mental illness, physical impairments, ataxia and sensory deficits. Experimental animal models suggest that TH influences proliferation of neuronal precursor cells and glia, axonal and dendritic morphogenesis, myelination and synaptogenesis in at least some brain regions. Despite these data, specific neurodevelopmental endpoints regulated by TH are not well defined. As a first step to addressing this critical data gap, we screened specific neurodevelopmental endpoints to identify those modulated by TH in primary neuron-glia co-cultures dissociated from cortices and hippocampi of the neonatal rat. Co-cultures were grown in varying concentrations (0-30 nM) of triiodothyronine (T3) or thyroxine (T4) prior to fixing and immunostaining for appropriate biomarkers to quantify axonal growth on day *in vitro* (DIV) 2, dendritic arborization on DIV 9, synaptogenesis on DIV 21 and glial cell proliferation and size on DIV 2, 7 and 21. T3 nor T4 significantly altered axonal or dendritic length in cortical or hippocampal cultures. While varying the TH concentration did not alter glial cell number, it did significantly alter glia cell size in a non-monotonic concentration-related fashion. These findings suggest that TH influences very specific neurodevelopmental endpoints in a region-dependent manner, which has significant implications for developing adverse outcome pathways that link TH disruption to adverse neurodevelopmental outcomes. Funding provided by US EPA grants R835550 and R833292, and NIH grant ES011269.

PS 1885 Effects of 2,2′,4,4′-Tetrabromodiphenyl ether (BDE 47) on Thyroxine Metabolism and Transport in Primary Rat and Human Hepatocytes

V. Richardson[2] and C. Mazur[1]. *[1]US EPA, Athens, GA and [2]US EPA, Research Triangle Park, NC.*

Polybrominated diphenyl ethers (PBDEs), a major class of brominated flame retardants, are used in consumer products including furniture, electronics, textiles, and plastics. PBDEs bioaccumulate in wildlife and humans; BDE 47 is the predominant PBDE congener detected and typically accounts for ~half of total tissue PBDEs. They act as endocrine disruptors through interference with thyroid hormone (TH) homeostasis. Although consequences of exposure to humans have not been determined, PBDE-mediated decreases in TH profoundly affect developing rodents. PBDE-mediated decreases in THs are linked to the induction of hepatic uridinediphosphate- glucuronosyltransferases (UGTs) in rodents. It is not certain that metabolism alone mediates the effects of PBDEs on circulating TH concentrations as PBDEs increase the expression of genes involved not only in TH metabolism but also TH transport. In this study, we used primary rat and human hepatocytes to examine the effects of BDE 47 on hepatic metabolism and uptake of thyroxine (T4) and the role these mechanisms may play in TH disruption. Hepatocytes from human or Sprague-Dawley rats were treated (72 hours) with BDE 47 (0 or 30 uM). Medium was removed and replaced with 0.05 or 0.1 uM T4 (rat or human median serum concentration, respectively). Cells were collected after 30 seconds to measure T4 uptake and media was collected after 24 hours to measure T4-glucuronide (T4G). Medium and cells were analyzed by liquid chromatography. In untreated hepatocytes, T4G in the medium of rats was ~20-times greater than in the medium of humans. Following BDE 47 exposure, T4G in the medium of rat hepatocytes was unchanged; however, T4G in the medium of human hepatocytes increased ~2-fold. T4 uptake into rat and human hepatocytes was unchanged following BDE 47 exposure. The data do not show significant BDE 47-mediated alteration in active transport of T4 in rat and human hepatocytes, but do show significant species differences in hepatic UGT activity toward T4. (This abstract does not reflect US EPA policy.)

PS 1886 Identification of Chemical Features Linked to Thyroperoxidase Inhibition

S. O. Simmons[3], E. D. Watt[3], K. Paul-Friedman[1], K. A. Houck[3], R. S. Judson[3], M. W. Hornung[2] and K. M. Crofton[3]. *[1]Bayer CorpScience LP, Morrisville, NC; [2]Office of Research and Development, US EPA, Duluth, MN and [3]Office of Research and Development, US EPA, Research Triangle Park, NC.*

Disruption of maternal serum thyroid hormone (TH) adversely affects fetal neurodevelopment. Therefore, assay development within the US EPA ToxCast program is ongoing to enable screening for chemicals that may disrupt TH, in support of the Endocrine Disruption Screening Program (EDSP21). The AUR-TPO assay was recently developed to screen >1,000 ToxCast chemicals for potential thyroperoxidase (TPO) inhibition activity. TPO is critical for TH synthesis and is a known target of thyroid-disrupting chemicals. The bioactivity results from the AUR-TPO assay were used to identify chemical substructures associated with *in vitro* TPO inhibition. Substructure profiles were generated for each chemical in the ToxCast test set using the publicly-available ToxPrint 2.0 chemotypes. Chemotypes enriched among the putative TPO inhibitors were identified using a cumulative hypergeometric probability ($p < 0.01$). Of the total 729 chemotypes evaluated, 31 were overrepresented among TPO inhibitors. Examination of those 31 chemotypes revealed four basic pharmacophores that accounted for 70% of the ToxCast chemicals active in the AUR-TPO assay: aromatic alcohols, aromatic amines, thiocarbonyls and phosphothioates. Chemico-structural analysis of AUR-TPO screening results enabled the identification of chemical features that likely drive TPO inhibition in the AUR-TPO assay. This highlights the potential to identify thyroid-disrupting chemicals *in silico* using structural alerts identified by chemotype analysis and confirmed by *in vitro* testing. The pharmacophores identified using this approach also offer key insights into mechanisms of TPO inhibition, which should strengthen the development of predictive tools. This abstract does not necessarily reflect the policy of the US EPA.

PS 1887 Potential Use of a Sodium/Iodide Symporter (NIS) Inhibition Assay for Thyroid Screening in FRTL Cells

A. R. Buckalew, A. S. Murr, D. R. Hallinger, T. E. Stoker and S. C. Laws. *National Health and Environmental Effects Research Laboratory, US EPA, Research Triangle Park, NC.*

The sodium/iodide symporter (NIS) is a transmembrane glycoprotein that facilitates active iodide uptake into thyroid follicular cells, which is essential for thyroid hormone synthesis. We are using the FRTL cell line (derived from Fischer rat thyroid) in an *in vitro* radioactive iodide uptake (RAIU) assay for environmental compounds that disrupt thyroid function. FRTL (ATCC CRL-1468) cells were cultured in 96 well plates (density of 4.5 x 104 cells/ml) for 3 days, exposed to test chemicals and assayed for 125I- uptake via RAIU. Chemical concentrations were evaluated in log-10 increments up to the limit for cytotoxicity or solubility. Cell viability was evaluated to differentiate between chemical inhibition and cytotoxicity. The dynamic range of the assay with 50uM sodium perchlorate ($NaClO_4$) was 12-fold and the IC50 for $NaClO_4$ agreed with published literature. Compounds known to inhibit NIS and inactive compounds (e.g., NaF) assayed matched relative inhibition potencies observed in two other cell lines being developed by EPA for thyroid screening (hNIS-HEK293 and hNIS-HEK293T-EPA). Although the FRTL cell line may not be suitable for high-throughput screening due to complex media requirements and slow growth, it should be useful as a low-medium throughput assay with physiological relevance. This abstract does not necessarily reflect EPA policy.

1888 Development of an *In Vitro* Radioactive Iodide Uptake Assay (RAIU) with Human NIS-Expressing HEK293T-EPA Cell Line

A. S. Murr, D. R. Hallinger, A. R. Buckalew, S. O. Simmons, T. E. Stoker and S. C. Laws. *National Center for Computational Toxicology, US EPA, Research Triangle Park, NC; National Health and Environmental Effects Research Laboratory, US EPA, Research Triangle Park, NC.*

Many high-throughput screening (HTPS) assays are available in the US EPA ToxCast program for estrogen and androgen pathways; only a limited number of assays exist for thyroid pathways. One potential target of thyroid-disrupting chemicals is the active uptake of iodide into the thyroid follicular cell via the sodium/iodide symporter (NIS), which is essential for the synthesis of thyroid hormones. Our laboratory evaluated a RAIU assay to screen for environmental chemicals that inhibit iodide uptake via NIS. We initially evaluated this assay with stably transfected hNIS-HEK293 cells (CEA, France) and then adapted it for use with a second-generation cell line, hNIS-HEK293T-EPA. These new cells stably express human NIS and efficiently uptake 125I-. In both cell lines, we optimized parameters such as cell density, incubation time, and 125I- exposure to ensure reliability (current Z'>0.8 0.08 for both cell lines) and sensitivity (dynamic range 63 fold and 220 fold, respectively) for detecting inhibitors of iodide uptake. IC_{50}; values were determined using nonlinear regression analyses of RAIU assay activity from cells exposed to multiple concentrations of each test compound. All response values were normalized to percent vehicle (HBSS, 10mM HEPES). The relative inhibition ranked by potency of the tested compounds (in both cell lines) are as follows: KPF_6 > $KClO_4$ > $NaClO_4$ > $NaBF_4$ >> NaSCN >>$NaNO_3$. NaF, a negative control, had no impact on iodide uptake. After test compound exposure, cells were evaluated for cytotoxicity to differentiate iodide uptake inhibition in viable cells from cell death. Based on their reliable performance in the RAIU assay, the hNIS-HEK293T-EPA will soon be used to screen ToxCast chemicals for inhibitors of iodide uptake via NIS. This abstract does not necessarily reflect EPA policy.

1889 Use of an *In Vitro* Radioactive Iodide Uptake (RAIU) Assay for High-Throughput Screening of ToxCast Chemicals at US EPA

D. R. Hallinger[2], A. S. Murr[2], A. R. Buckalew[2], S. O. Simmons[2], S. C. Laws[2] and T. E. Stoker[2]. [1]*National Center for Computational Toxicology, US EPA, Research Triangle Park, NC and* [2]*National Health and Environmental Effects Research Laboratory, US EPA, Research Triangle Park, NC.*

Active transport of I- into thyroid follicular cells via the Na+/I- symporter (NIS) is essential for the biosynthesis of thyroid hormones T3 and T4. Iodide uptake into the thyroid gland is known to be disrupted by environmental contaminants including perchlorate, thiocyanate, and nitrate. In support of the Endocrine Disruptor Screening Program for the 21st century (EDSP21), a robust *in vitro* RAIU assay (Z'>0.8± 0.02) was coupled with a newly-generated human NIS-expressing HEK293T cell line (hNIS-HEK293T-EPA) to identify chemicals that inhibit cellular 125I- uptake via NIS. To assess the feasibility of using RAIU in hNIS-HEK293T-EPA cells as a high-throughput screening assay, a small-scale screening exercise was conducted. Inhibition of 125I- uptake was evaluated in a training set of 20 chemicals which included some known to suppress serum thyroid hormone concentrations in EDSP Tier 1 *in vivo* assays. Chemicals were tested in a multi-concentration format up to 100µM. Fifteen of the 20 test chemicals (75%) inhibited 125I- uptake by ≥ 20%, the threshold used to indicate an active response. A cell viability assay was performed in parallel to ensure that loss of iodide uptake could not be attributed to cytotoxicity. Chemicals were ranked by a differential of RAIU and cytotoxicity EC20 values to determine chemical selectivity for NIS inhibition. Of the 15 actives, 4 (27%) were highly selective for NIS inhibition. Efforts are underway to automate the assay and increase throughput to enable screening of the Phase I/II ToxCast chemical libraries for thyroid-disrupting chemicals that inhibit T3/T4 synthesis via NIS. This abstract does not necessarily reflect US EPA policy.

1890 Establishment of Sensitive, Quantitative and Real-Time Cellular Assays for Detection of Modulators for Human Thyroid Hormone Receptor

C. Jin, Y. Abassi, B. Choi, X. Wang and X. Xu. *ACEA Biosciences, Inc, San Diego, CA.*

Thyroid Hormone receptor (THR) plays critical roles in development and the regulation of metabolism. Using real time impedance technology, cell based kinetic assays for detection of THR modulators were developed. One human prostate cancer cell line LNCaP, and a rat pituitary tumor cell line GH3, were used for the study. Stimulation of these cells with thyroid hormones such as triiodothyronine (T3) and thyroxine (T4), lead to alterations in cell number and cell adhesion, which can be detected by gold microelectrodes embedded in the bottom of the well of specialized microelectronic plates. The observed EC50 values using human cell line LNCaP are comparable to existing *in vitro* cell based reporter assay systems, with EC50 for T3 around 0.2nM, and for T4 around 20nM. The observed EC50 values using rat cell line GH3 are the most sensitive compared to other method, with EC50 for T3 around 20pM, and for T4 around 2nM. The specificity of the assays was established using well characterized THR antagonists 1-850 and desethylamiodarone. LNCaP cells were previous reported to respond to modulators of estrogen and androgen receptor (ER and AR). Using antagonists against different hormone receptors, hormones targeting AR can be differentiated from those targeting TR and ER. While with GH3 cells, the response profiles for estrogen and thyroid are different. Altogether, the data suggests that the impedance based real time cellular assays with the 2 cell lines have the capacity to sensitively, selectively and quantitatively detect endogenous THR responses. The information can be further developed to understand interaction among different hormone signaling pathways, and to detect chemicals interfering with thyroid hormone synthesis and metabolisms.

1891 Integration of Relevant Carcinogenicity and Endocrine Data in a Systemic Approach Establishes the Mode of Action for Pronamide Induced Thyroid Tumors

J. LaRocca[1], S. Marty[2], S. Papineni[1], M. J. LeBaron[2] and R. J. Rasoulpour[1]. [1]*Dow AgroSciences, Indianapolis, IN and* [2]*The Dow Chemical Company, Midland, MI.*

Chronic exposure to the herbicide pronamide (propyzamide) results in an increased incidence of thyroid follicular cell tumors (adenomas) in Crl:CD(BR) rats. The incidence of thyroid follicular cell adenomas, out of 50/group, was 4, 2, 6 and 16 in male rats and 1, 2, 1 and 7 in female rats given 0, 40, 200 and 1000 ppm pronamide in the diet, respectively. As pronamide is not genotoxic, the hypothesis for pronamide-induced thyroid tumors is an indirect effect on thyroid hormone imbalance, which is characterized by six key events, 1) hepatic enzyme induction, 2) increased hepatic metabolism T4, 3) decreased serum T4, 4) Stimulation of HPT axis, 5) increased TSH concentration, and 6) increased thyroid weight and follicular cell hyperplasia. Evaluation of data from a series of regulatory guideline and mechanistic studies aimed at identifying the causative and associated key events helped support this mode of action (MoA). Data was also generated for pronamide for Tier 1 screening in the Endocrine Disruptor Screening Program (EDSP). Upon evaluation, several endpoints from the Tier 1 screening battery proved relevant and necessary for incorporation into the pronamide mode of action/human relevance framework. Specifically, liver weight, thyroid weight, thyroid histopathology, and thyroid hormone data from the male and pubertal female assays added support to the weight-of-evidence for the proposed MoA. For instance, in the pubertal assays terminal serum T4 levels were decreased by 25–48% in females at doses ranging from 25–300 mg/kg/day and 13–23% in males at doses ranging from 10–25 mg/kg/day, which was critical to establish key event # 3. Data from the pubertal assays also supported key events #1 (liver weight), #5 (TSH concentrations), and #6 (thyroid weight). These results demonstrate that integration of all available relevant data from several different studies into lines of evidence was essential to confidently establish the MoA for pronamide-induced thyroid tumors.

1892 Identification of Molecular, Cellular and Behavioral Endpoints Associated with Developmental Hyperthyroidism and Hypothyroidism in Larval Zebrafish

K. M. Walter, G. W. Miller, K. Hayakawa, X. Chen, B. Puschner and P. J. Lein. *Department of Molecular Biosciences, University of California Davis, Davis, CA.*

Thyroid hormones (THs) are critical for proper fetal neurodevelopment, influencing multiple neurodevelopmental processes including the differentiation and maturation of oligodendrocytes. Many environmental contaminants can disrupt TH-signaling, and it is widely postulated that these contaminants cause developmental neurotoxicity (DNT) through disruption of TH-mediated signaling. However, the identification of mechanisms linking chemical TH disruptors to altered neurodevelopment has been limited by gaps in the understanding of how THs regulate neurodevelopment and the identification of specific neurodevelopmental endpoints directly affected by THs. To address these gaps, we are characterizing the phenotypes associated with developmental hyperthyroidism and hypothyroidism in a larval zebrafish model using pharmacological treatment with T4 and T3 and a conditional thyroid ablation line. Endpoints examined include the spatiotemporal expression profiles of transcripts encoding transporters, deiodinases and thyroid hormone receptors (TRs), larval swimming behavior, and oligodendrocyte differentiation. The expression of three TH signaling components, deiodinase 3b, TRβ, and TH transporter MCT8, were significantly upregulated by T4 and T3 treatment, while some genes previously shown to be TH-responsive in rodents were unchanged. Additional preliminary results indicate that exogenous TH exposure impairs larval swimming behavior at time points and concentrations that do not exert overt teratological effects. The identification of endpoints that are indicative of TH-disruption during neurodevelopment will contribute to a better understanding of the roles of THs in neurodevelopment and establish the larval zebrafish as a model for screening environmental TH-disrupting chemicals for DNT risk. Supported by the USEPA (grant R83550 to PJL) and the NIEHS (T32 ES007059 predoctoral fellowship to KMW).

1893a Estrogen Activity Determinations of Ethanol Extracts of Seven Hair Relaxers in Breast Cancer Cell Bioassays

T. Mitchell[1], G. Guirard[1], S. Cureaux[1], P. Ma[1], L. Stiel[2], S. Montgomery[3] and T. Wiese[1]. *[1]College of Pharmacy, Xavier University of Louisiana, New Orleans, LA; [2]Department of Social Work & Social Ecology, Loma Linda University, San Bernardino, CA and [3]School of Behavioral Health, Loma Linda University, Loma Linda, CA.* Sponsor: K. Coleman.

Hair relaxers that contain sodium hydroxide or guanidine to break sulfhydryl bonds in hair proteins are widely used by African American women. With the recent suggestion that the use of hair relaxers could be associated with breast cancer disparities in African American women, it is of interest to examine the estrogen activity of these products. In this study, we test the hypothesis that the ethanol extracts of seven hair relaxers will induce estrogen agonist or antagonist activity in breast cancer cells in culture. Ethanol extracts (1 gm/1 ml) were prepared of the hair relaxer products: Life and Body, Nairobi, Design Essential, Affirm, Dr. Miracle, ORS Olive Oil, and African Pride. The estrogen agonist and antagonist activity of each extract was determined using the T47dkbLuc estrogen responsive reporter assay and the MCF-7 estrogen responsive proliferation assay. While the initial extracts were shown to be toxic to the breast cancer cells, 1/10 dilutions of the extracts induced no obvious toxic effects and were used for estrogen activity determinations. None of the extracts induced estrogen agonist activity in either bioassay. Four extracts displayed a dose-dependent, partial antagonist activity in both t47DkbLuc and MCF-7 cells. Since the antiestrogen activity of these four extracts could be rescued with high dose estradiol, the observed effect is likely estrogen receptor mediated and not due to toxic or other nonspecific effects. While hair relaxers have inherent dose-dependent cytotoxic effects due to the caustic and corrosive chemicals that modify protein structure to straighten hair, any estrogen mediated endocrine disrupting potential may be limited to the estrogen antagonist activity of some products. Identification of individual product components responsible for the observed antiestrogen activity, exposure levels in those using these products, and whether endocrine disruption would manifest in the consumer are yet to be determined.

1893 An F1-Extended Two Generation Reproductive Toxicity Study to Evaluate the Dietary Effects of Carbendazim on Thyroid Glands and its Hormones in Wistar Rats

S. K. Bommegowda[4], R. G. Gilmore[4], R. Read[4], J. C. Seely[1], A. Krygsman[2], J. J. Wrubel[3] and D. S. Dandekar[4]. *[1]Pathology, EPL-Inc., Research Triangle Park, NC; [2]Product Registration, Troy Corporation, Florham Park, NJ; [3]Regulatory Affairs, Nisso America Inc., New York, NY and [4]Toxicology, Xenometrics LLC, Stilwell, KS.*

Carbendazim is a broad-spectrum benzimadazole antifungal compound with potential antimitotic and antineoplastic activities. It may also be used as a preservative in paint, textile, papermaking and leather industry, as well as preservatives of fruits. In prior sub-chronic toxicity studies, gavage dosing of carbendazim to rats has led to thyroid toxicity. The purpose of this study was to determine the potential intergenerational dietary effects of carbendazim on thyroid glands (TG) and the level of thyroid hormones in Wistar rats (young adults and pups). In this study, daily dietary doses of 0, 250, 1000 and 2000 ppm carbendazim were administered to male and female rats of Parental generation, F1 generation (Cohort 1a, Cohort 1b, and Cohort 3), and F2 generation (Cohort 1b - PND 23 and PND 45 pups). At the end of the experiment, TSH, T4, and/or T3 levels were analyzed. TGs were collected, weighed and processed for histopathology evaluation. For TG semi-quantitative microscopic scoring, thyroid glands from all animals/sex/group (including thyroid glands from 12 animals/sex/group sampled for thyroid hormone analyses) were evaluated, using the guidance specified in the Tier 1 EDSP Pubertal protocol (OPPTS 890.1450): Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats. There were no differences between the control and carbendazim-treated group of rats in serum TG hormone levels. No exposure-related organ weight or histopathology changes were observed in TG of treated rats. There were no carbendazim-related changes in the TG semi-quantitative microscopic scoring (follicular cell height and colloid area) at any of the dietary levels tested. These results suggest that intergenerational dietary carbendazim exposure up to 2000 ppm had no effects on TG of Wistar rats.

1894 Immune Antibody and Cytokine Profiles Differentiate Behavior Phenotypes

D. A. Lawrence[2], T. K. Mondal[2], A. Mendoza[2] and L. Kearsing[1]. *[1]Center for Disability Services, Albany, NY and [2]Immunology, Wadsworth Center/NYS Dept Health, Albany, NY.*

Luminex and grating coupled surface plasmon coupled fluorescence (GCSPCF) technologies were used to screen samples from humans and mice. The human samples were obtained from newborn dried blood spots (NDBSs) collected from babies born in New York and later diagnosed with autism spectrum disorder (ASD) or typical development. The proteins eluted from the NDBSs were compared to plasma protein differences from the BTBR T+Itpr3tf/J (BTBR) mouse strain with ASD-like behavior and the C57BL/6J (B6) strain, which has normal behavior. BTBR mice have reduced social interactions, impaired play, low exploratory behavior, unusual vocalizations and high anxiety; they also have substantial depletion of the corpus callosum and severely reduced hippocampal commissure (Jackson Laboratory). The human and mouse comparisons demonstrate that humans and mice with different behaviors have different antibody and cytokine profiles from the control populations. Human antibodies from the NDBSs of babies that developed ASD and those from BTBR mice to brain antigens from B6 mice were assessed by GCSPCF and Western blot analysis and shown to differ from the antibodies specificities and isotypes from humans and mice with typical development and usually normal behaviors. The cytokine profiles of children with ASD and mice with ASD-like behavior also differed from controls.

1895 *In Utero* Exposure to 2-Aminoanthracene and Type-1 Diabetes

C. A. Mays, D. A. Hunter and W. E. Gato. *Chemistry, Georgia Southern University, Statesboro, GA.*

A recent diabetes report revealed an increased incidence in diabetes including type 1-diabetes (T1D). The increase in the numbers of T1D incidences are thought to be related to environmental reasons such as the exposure to environmental chemicals including arylamine 2-aminoanthracene (2AA). T1D is an inflammatory disease of the pancreatic islet in which insulin-producing beta cells are destroyed by auto-reactive T-cells and monocytic cells. The purpose of this study is to examine the extent to which 2AA exposure contributes to T1D. In this proposed study pregnant Sprague Dawley dams were exposed to different concentrations of

2AA through their diet from gestation through postnatal period. Certain genes that are cytokines and adipokines previously noted to play a significant role in inflammatory response were analyzed in the pancreas of the pups for alteration. Results showed the over-expression of pro-inflammatory protein IL-6. Up-regulation of humoral genes IL-7 and IL-21 were also noted. mRNA expression levels of the rest of the proteins were not significantly changed. Pathologic characterization showed no significant changes in the pancreas across control and treated groups. However, reduction in pancreatic islets of the high dose group indicated a loss of cell mass. The study is still ongoing to characterize glucose, insulin and level of inflammation. Furthermore, IL-6 protein will be quantified via immunoblotting technique. It appears, *in utero* exposure to the PAH, 2AA increases susceptibility to T1D.

PS 1896 Effect of Aspirin on Glucose Tolerance and Pancreatic Endocrine Function in Type 2 Diabetic Rats

H. Raza[3], L. Amiri[3], A. John[3], J. Shafarin[3], E. Adeghate[3] and F. C. Howarth[3]. *[1]Department of Anatomy, CMHS, UAE University, Al Ain, United Arab Emirates; [2]Department of Biochemistry, CMHS, UAE University, Al Ain, United Arab Emirates and [3]Department of Physiology, CMHS, UAE University, Al Ain, United Arab Emirates.*

Type 2 diabetes is the most common metabolic disorder associated with impaired pancreatic function and insulin resistance. The metabolic complications in type 2 diabetes include hyperglycemia, hyperlipidemia, hyperinsulinemia, and reduced insulin response. Aspirin (ASA) has been implicated with the prevention of diabetes and cardiovascular disorders. In this study, five month old Goto-Kakizaki (GK) rats with mild hyperglycemia (fasting blood glucose 80-95 mg/dl vs 55-60 mg/dl Wistar control rats) were used. Two subgroups of GK and Wistar control rats were injected intraperitoneally with 100mg ASA/kg body weight/day for 5 weeks, following which the animals were sacrificed and blood and tissues collected, after performing glucose tolerance (2 h post 2g IP glucose ingestion) tests. Our results showed that ASA treatment caused a moderate decrease in hyperglycemia. However, a significant improvement in glucose tolerance was observed in GK rats compared to the control rats. Also, the ASA treated GK rats exhibited a significant decrease in blood insulin level. ASA treatment caused a marked reduction in the pro-inflammatory PGE2, which was markedly higher in GK rats. No significant organ toxicity was observed after ASA treatment at this dose and time period. However, the total cholesterol and lipoprotein levels which were significantly increased in GK rats, decreased after ASA treatment. ASA also caused alterations in metabolic and oxidative stress associated complications in diabetic rat tissues. Microscopic studies on pancreatic cells showed improved beta-cell structural and functional integrity in ASA-treated rats which was confirmed by SDS-PAGE and Western blot analysis. The improved glucose tolerance in ASA-treated GK rats may be associated with the anti-inflammatory properties of ASA which increased insulin responses and energy utilization in target tissues. These results may have implications in determining the therapeutic use of ASA in insulin-resistant type 2 diabetes.

PS 1897 Genistein Modulation of Type 1 Diabetes (T1D) in Non-Obese Diabetic (NOD) Mice Following Developmental or Adult Exposure

G. Huang and T. L. Guo. *UGA, Athens, GA.*

Despite many hypothesized benefits of isoflavone genistein (GEN) from soy-based products, many questions surrounding GEN's immunotoxic effects have yet to be answered. The widely used non-obese diabetic (NOD) mice spontaneously develop autoimmune pancreatitis and, subsequently, diabetes that shares many immunogenetic features with human T1D. Females have a higher percentage (80%) to become diabetic by 6 months of age comparing to 20-30% in the males. In this study, adult NOD male and female mice were gavaged with GEN at the dose level of 20 mg/kg for 5 months; and it was found that over time, the blood glucose levels in both male and female were not significantly different from the vehicle control group, although the female mice exposed to GEN demonstrated a slight delay of diabetic onset compared to the control group. The glucose tolerance test revealed a significant decrease in blood glucose levels (both fasting and the 2-h time point) in males following GEN exposure. To determine if developmental exposure to GEN could affect the incidence of T1D, NOD mice were exposed to GEN by maternal gavage (20 mg/kg) from gestation day 7 to the day of weaning at postnatal day (PND) 21. In female NOD offspring, perinatal exposure to GEN significantly increased the incidence of T1D at PND 113 (24% vs. 3%), 120 (24% vs. 6%), 147 (39% vs. 16%) and 162 (45% vs. 23%), while exacerbating T1D at PND 113 (33% vs. 4%), 120 (33% vs. 8%), 125 (38% vs. 12%), 147 (54% vs. 20%), 162 (63% vs. 28%) and 168 (67% vs. 36%). Serum levels of IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p40, IL-12p70, MCP-1 and TNF-α were measured, and T1D exacerbation was associated with a decrease in IL-10 level in female offspring. In male NOD offspring, the incidence of T1D was numerically decreased following perinatal exposure to GEN. Flow cytometric analysis suggested that both the %T cells and %CD4+ T cells were significantly decreased following developmental GEN exposure in males. Among the 11 cytokines and chemokines analyzed, serum level of IL-1α was reduced in GEN-exposed male NOD offspring. It has been reported that IL-1 receptor deficiency can slow the progression to diabetes in the NOD mice, suggesting that GEN reduction of IL-1α might be a novel mechanism of action. The differential effects of GEN on blood glucose levels in male and female NOD mice suggest that the estrogenic properties of this compound may contribute to its modulation of T1D. Furthermore, the effects of GEN are dependent on the window (fetus vs. adult) of exposure (Supported by NIH R21ES24487).

PS 1898 Blockade of CB1 Cannabinoid Receptors is Associated with Growth of the Gut Bacterium *Akkermansia muciniphila* and Attenuation of Diet-Induced Obesity

P. Mehrpouya-Bahrami[1], P. Nagarkatti[1] and M. Nagarkatti[2]. *[1]Department of Pathology ,Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia, SC and [2]Department of Pathology ,Microbiology and Immunology, School of Medicine, University of South Carolina,and WJB Dorn VA Medical Center, Columbia, SC.*

Obesity is characterized by chronic low-grade, systematic inflammation, altered gut microbiota and gut barrier disruption. The endocannabinoid-system (eCB) plays a major role in the regulation of the inflammation and metabolic disorders associated with obesity. We investigated the effect of treatment of mice with SR141716A, a cannabinoid receptor1 (CB1) antagonist, on Diet-Induced Obesity (DIO), specifically whether such a treatment can induce anti-inflammatory state in adipose tissue. We demonstrated that blockade of CB1 receptor reduced plasma LPS level, circulating inflammatory cytokines and the trafficking of M1 macrophages into the adipose tissue. This decreased inflammatory tone was associated with a lower intestinal permeability and improved hyperglycemia and insulin resistance. In order to better understand the beneficial effects of SR141716A in DIO model, we performed 16S rRNA metagenomic sequencing to investigate alterations in the gut microbiome. Analysis of fecal samples revealed that treatment with SR141716A dramatically increased the relative abundance of *Akkermansia muciniphila* within the *Verrucomicrobia phylum*. Although an increase in the genus *Prevotella* also emerged as strong biomarkers of SR141716A treatment, the increase in *A. muciniphila* was the only strong biomarker consistently detectable across taxonomic levels and sample type. SR141716A treatment counteracted the thickness reduction of mucosal layer in high-fat diet fed mice. We showed that both eCB system and gut microbiota regulates adipogenesis, by increasing markers of adipocyte differentiation (PPAR-γ, C/EBP-α), lipogenesis (ACC1, FASN) and lipid oxidation (CPT1, ACOX1, PGC1α, and PPARα). Our data suggest that blocking of CB1 receptors ameliorates obesity by restoring *A. muciniphila* abundancy in gut. (Supported by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357).

PS 1899 Alteration of the Gut Microbiota with Oral Broad-Spectrum Antibiotics Alleviates the Severity of Autoimmune Uveitis Through Modulation of Effector T Cell Subsets

Y. K. Nakamura, C. Metea, M. Asquith, H. Gruner, Y. Nakamura, C. Moscibrocki, J. T. Rosenbaum and P. Lin. *Oregon Health and Science University, Portland, OR.*

Recent studies have shown the importance of the gut microbiome in the development of various immune-mediated diseases. We previously reported that the severity of experimental autoimmune uveitis (EAU) in mice was alleviated with oral broad-spectrum antibiotics partly by inducing regulatory T cell (Treg) populations. The purpose of this study was to investigate how alteration of the gut microbiota with oral antibiotics affects effector T cell (Teff) populations and the gut bacterial composition. Female B10.RIII mice were immunized to induce EAU. Mice were treated with an oral antibiotic cocktail or individual antibiotic, starting 1 week prior to antigen challenge. Lymphoid tissues and eyes were collected at 1 to 4-week post-immunization. We performed clinical EAU grading, quantification of T cell populations and cytokines, and bacterial phyla/class qPCR. Statistical analysis was performed using Mann-Whitney U test. There was differential regulation of Th1 and Th17 cell frequencies at 1-week post-immunization prior to the onset of oc-

ular inflammation in extraintestinal lymphoid tissues of the antibiotic group, compared to the control group. Similarly, lower levels of inflammatory cytokines were found in these tissues of the antibiotic group. We also observed significant differences in the abundance of selected bacterial phyla/class between these two groups as early as 1-week post-immunization. These coincided with attenuation of clinical EAU scores and up-regulation of Treg cells. Individual administration of metronidazole or vancomycin showed similar effects on Treg and Teff cells, as well as clinical EAU scores. Alteration of gut microbiota with oral broad-spectrum antibiotics appears to alleviate the severity of EAU by down-regulating Teff cell differentiation and inflammatory cytokine production at extraintestinal sites even prior to the onset of clinical ocular inflammation, in addition to up-regulating Treg cell development at intestinal/extraintestinal sites. Furthermore, it seems to be associated with changes in gut bacterial phyla/class composition starting shortly after antibiotic administration. Our findings suggest novel therapeutic potential for extraintestinal immune-mediated diseases, such as autoimmune uveitis, by targeting the gut microbiota.

PS 1900 Indole-3-Carbinol Ameliorates Murine Colitis Symptoms Through Alterations in Gut Microbial Composition by Restoring Firmicute-Associated Species and Decreasing Colitis-Associated Bacteriodetes Species

P. B. Busbee[1], M. Nagarkatti[1] and P. Nagarkatti[2]. [1]*Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC and* [2]*Vice President for Research, University of South Carolina, Columbia, SC.*

Colitis is an autoimmune disease characterized by acute or chronic inflammation of the large intestine. Currently there is no cure for patients suffering from colitis, and most treatments involve the use of immunosuppressive drugs that can have adverse side-effects or increased toxicity. In the current study, we investigated the effects of indole-3-carbinol (I3C), a component found in a number of cruciferous vegetables, on two murine colitis models (DSS and TNBS). Our data shows that administration of I3C alleviates symptoms associated with colitis in both of these models, which includes reversal of weight loss and colon shortening. In addition, mice treated with I3C showed decreased levels of circulating inflammatory serum cytokines and cellular infiltration into the colon. In order to better understand the beneficial effects of I3C against colitis, we performed 16S rRNA metagenomic sequencing to investigate alterations in the gut microbiome after induction of colitis by TNBS and treatment with I3C. Analysis of cecal flushes revealed that TNBS administration leads to a decrease in several Firmicute species (e.g. *Sedimentibacter hydroxybenzoicus* and *Clostridium frigoris*), while increasing several species belonging to the *Phylum bacteriodetes* (e.g. *Bacteroides acidifaciens*, *Dysgonomonas wimpennyi*, and *Bacteroides xylanisolvens*). However, mice that were treated with I3C showed a remarkable reversal in these gut microbial alterations caused by TNBS colitis induction, having gut microbiome similar to that of vehicle-treated control mice. Collectively, these data suggest that I3C is able to ameliorate colitis by preventing pathogenic gut microbial dysbiosis and restoring gut microbiome composition to a more homeostatic state. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357).

PS 1901 Fibroblast Growth Factor 21 Deficiency Attenuates DSS-Induced Acute Colitis through Activation of Intestinal Epithelial Cell Stat3-Mediated Pathway

L. Liu[1,2], C. Zhao[2], G. Dryden[2], C. McClain[2] and W. Feng[2]. [1]*College of Animal Science and Technology, Jilin Agricultural Science and Technology University, Jilin, China and* [2]*Department of Medicine, Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Background: Fibroblast growth factor 21 (FGF21) is a novel metabolic regulator in glucose and lipid metabolism. Recent studies showed that FGF21 is also induced by inflammatory stimuli. However, its role in inflammatory bowel disease (IBD) has not been examined. Methods: Experimental IBD model was induced by adding 2.5% (wt/vol) dextran sulfate sodium (DSS) into drinking water for 3, 5 and 7 days, respectively, in FGF21 knockout (KO) and wide type (WT) mice. Mouse colon tissues were analyzed for colitis severity and inflammatory cell infiltration. FGF21 and inflammatory makers were determined in serum and tissues samples. The T84 intestinal epithelial cell line was used for additional *in vitro* studies. Results: In WT mice treated with DSS induced an elevation in serum FGF21 and a significant body weight loss in a time dependent manner. Gene expression studies showed that liver and epididymal white adipose tissues were the major sources of circulation FGF21. Surprisingly, the body weight loss and colitis severity were significantly attenuated in FGF21 KO mice. Further analysis showed that FGF21 KO mice had less colon shortening, histological damage, less neutrophil infiltration compared to WT mice. FGF21 deficiency decreased DSS treatment-increased serum concentrations of IL-6, TNF-α and IL-1β and their mRNA levels in colon tissues. Colonic epithelial cell proliferation, examined by BrdU staining, was increased in the KO mice. DSS treatment caused a decrease in epithelial cell proliferation and Paneth and Goblet cell numbers in WT mice, which were attenuated in the KO mice. Importantly, FGF21 depletion dramatically increased signal transducer and activator of transcription-3 (STAT3) activation in intestinal epithelial cells, along with a decreased suppressor of cytokine signaling-3 (Socs3) expression. FGF21 pretreatment inhibited IL6-induced STAT3 phosphorylation in human intestine epithelial T84 cells. Conclusion: Circulating levels of FGF21 were increased in acute colitis in mice. FGF21 deficiency attenuated DSS-induced acute colitis, which is likely mediated by the inhibitory effect of FGF21 on IL-6-induced-STAT3 activation in intestinal epithelial cells.

PS 1902 CYP2E1-Mediated Oxidative Stress Regulates MyD88 Expression and Function in Toxin Potentiation of Nonalcoholic Steatohepatitis

S. Das[4], D. Dattaroy[4], F. Alhasson[4], V. Chandrashekaran[4], R. Seth[4], G. Michelotti[2], A. M. Diehl[1], M. Nagarkatti[4], P. Nagarkatti[4] and S. Chatterjee[4]. [1]*Duke University, Durham, NC;* [2]*Metabolon, Inc., Durham, NC;* [3]*University of South Carolina, Columbia, SC and* [4]*Pathology and Microbiology, University of South Carolina, Columbia, SC.*

Extending our previous findings of peroxynitrite-induced TLR4 trafficking in toxin-potentiation of NASH, we test the hypothesis that CYP2E1-mediated oxidative stress and its downstream HMGB1 release regulates MyD88 expression which is critical for innate immune activation in toxin potentiation of NASH. Using a high fat (60%kcal)-fed NASH model (baseline obesity), where bromodichloromethane was used as a second hit, we showed that there was a significant increase in MyD88 mRNA and protein in NASH mice while the levels were significantly decreased in CYP2E1 KO and Diallyl sulfide-treated (CYP2E1 inhibitor) groups. HMGB1 levels were significantly higher in NASH group as compared to only high fat -fed group and CYP2E1 KO or DAS-treated group suggesting a strong correlation. Higher HMGB1 and higher MyD88 also correlated strongly with NASH progression, higher serum ALT, hepatocellular necrosis, ballooning and inflammation while MyD88 KO mice receiving identical exposure to diet and CYP2E1 activator showed significant decrease in these symptoms. Mechanistically, immortalized Kupffer cells treated with HMGB1 and leptin (representing strong leptin resistance) showed a significant increase in MyD88 and 3-nitrotyrosine formation (immunohistochemistry) while apocynin or FeTPPS blocked the increase suggesting a possible role of NADPH oxidase mediated peroxynitrite. To show the role of oxidative stress and activation of PI3K pathway, HMGB1-primed cells were treated with PI3/AKT pathway inhibitor LY294002. Results showed that LY294002 significantly inhibited MyD88 levels, CD68 expression and MCP1 release suggesting a strong role of PI3/AKT pathway in redox stress mediated MyD88 expression and Kupffer cell activation. In conclusion we report a novel role of redox stress mediated MyD88 induction in NASH pathogenesis that might be complementary to the TLR4 pathway.

PS 1903 Differing Pulmonary Immune Responses Following Exposure to Thoracic Radiation in Fibrosis Sensitive and Resistant Mice

A. M. Groves[2], C. J. Johnston[2], R. S. Misra[2], T. A. Beach[2], J. P. Williams[2] and J. N. Finkelstein[2]. [1]*Environmental Medicine, University of Rochester, Rochester, NY and* [2]*Pediatrics, University of Rochester, Rochester, NY.*

The lung is a radiation sensitive organ. Exposure to thoracic radiation (RT) results in early acute injury followed by inflammatory responses and microenvironmental alterations that promote the development of pneumonitis and pulmonary fibrosis. Activated macrophages (MP) may contribute to the development of fibrosis in the lung, becoming phenotypically polarized to orchestrate inflammatory responses. T cells have also been found to accumulate in the lung following RT and are master regulators of inflammation. The role of these cells in RT induced pulmonary fibrosis was investigated. We hypothesized that RT would lead to lasting alterations in the pulmonary immune microenvironment. To assess this, lung digests and mediastinal lymph nodes (MLNs) were collected at various time points following exposure from fibrosis prone C57Bl/6J (C57) and resistant C3H/HeJ (C3H) mice exposed to 0 Gy or

12.5 Gy thoracic RT. CD45+ leukocytes were characterized by flow cytometry. Alveolar, interstitial, and infiltrating MP were identified in the lung and markers of activation phenotypes were assessed in each population. T helper cells and regulatory T cells (Tregs) were characterized in MLNs. RT decreased numbers of alveolar MP and MLN T cells at 3 wk post RT in both C57 and C3H mice. In contrast, numbers of interstitial and infiltrating MP increased. Although loss of expression of CD206, a marker of alternative activation, was noted in alveolar MP of both C3H and C57 mice at 3 wk post RT, an increase in this marker was observed in interstitial and infiltrating MP of C3H mice through 16 wk post RT, with this response being abrogated in C57 mice. In contrast, expression of proinflammatory Ly6C by infiltrating MP, and IFN-γ by T helper cells was greater in C57 than C3H mice, while expression of immunosuppressive Foxp3 by Tregs was decreased by 16 wk following RT in C57 mice, suggesting the immunosuppressive capacity of this population was impaired. Taken together, these results suggest RT leads to losses in the capacity of T cells to effectively regulate inflammation, while differences in the responses of lung MP populations between the fibrosis-prone and resistant mice suggest a possible role for these cells in the development of long-term consequences of pulmonary RT. Funded By: R01 AI101732-01, U19AI091036, P30 ES-01247 and ES T32 07026

1904 Investigating the Role of B Cells in the Pathogenesis of Hydrocarbon Oil-Induced Diffuse Pulmonary Hemorrhage

P. Prasad[1,3], A. Chung[1], I. Valera[1], R. Sato[1], M. C. Fishbein[3] and R. R. Singh[1]. *[1]Division of Rheumatology-Dept. of Medicine, UCLA David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA; [2]Interdepartmental Doctoral Program in Molecular Toxicology, UCLA, Los Angeles, CA and [3]Pathology and Laboratory Medicine, UCLA, Los Angeles, CA.*

Background: Humans are exposed to hydrocarbon oils via inhalation (exhaust fumes from cars/oil refineries), dermal contact (cosmetics), and ingestion (food, laxatives). Hydrocarbon oil 2,6,10,14-tetramethylpentadecane (TMPD) is present in crude/mineral oils, petroleum, plants and marine organisms. A community study suggested an increased risk of inflammatory diseases in individuals exposed to TMPD. TMPD also induces inflammatory diseases in animals including diffuse pulmonary hemorrhage (DPH) that pathologically mimics DPH in humans. DPH occurs in patients with exposure to toxic substances (pesticides), recreational drugs (cocaine), chemotherapeutics, inflammatory disorders, and organ transplantation. It has no effective treatment and is often fatal. Results: We found that compared to none of the controls [control oil (n-Hexadecane) or PBS], 72% of TMPD-exposed C57BL/6 (B6) mice developed DPH/pneumonitis and infiltration of multiple immune cell types in the lungs at 2-4 weeks of exposure. We tested the hypothesis that TMPD exposure causes a rapid expansion and activation of innate B cell types in B6 mice. At 1-week timepoint, prior to any histopathological changes, while both Hexadecane and TMPD caused abnormalities of myeloid cells, only TMPD caused increased infiltration with B1 B cells (CD19+CD11b+/CD19+CD5+) in the lungs. The proportions and numbers of these B1 B cells were simultaneously reduced significantly in their usual location, i.e., peritoneal cavity in TMPD-exposed mice. Global gene expression analyses in the lungs of these mice revealed a differential expression of a cohort of immune-inflammatory genes including a chemokine Cxcl13 that drives the migration of B1 B-cells. Summary: These data guide us to posit that TMPD induces early changes in immune-response genes that drive the trafficking of innate B1 B cells to the lungs, where they play important roles in the pathogenesis of DPH. Ongoing experiments are focused to elucidate mechanisms by which B1 B cells are involved in DPH pathogenesis by adoptive transfer of B1 B cells from Green Fluorescent Protein (GFP) B6 mice injected with TMPD, which may induce DPH in naïve B6 recipients.

1905 Immune Responses in Co-Culture of Human Lung Cells (BEAS 2B) and Human PBMC's After Puerto Rican Airborne PM10 Exposure

N. E. Crespo. *Biochemistry, University of Puerto Rico, San Juan, PR.*

The Puerto Rican population exhibits the highest prevalence of asthma than any other racial or ethnic group in the US. Asthma is a chronic inflammatory disease of the respiratory airways, which can be triggered by a number of different environmental stimuli such as dust, fungus, pollen, endotoxins and particle pollution (PM). PM10 material may contain hazardous chemicals such like Phthalates, Trace Metals, Morpholines, PAHs and Benzamides, which can induce both oxidative, and inflammation responses. Some PM10 constituents remain constant yearly; others vary with dry air (summer) and humid air (fall) and local human activity (Rural/Urban), thus exacerbating asthma in patients. We believe that different sites produce different PM10 material during the year and induce different immune mediators, which may finally activate adaptive response by specific T cell differentiation. PM10 quartz filters were obtained from the PREQB. Organic extracts were prepared using a mixture of 5 mL - acetone and hexane 1:1 and extracted by Microwave and sohlex extraction. Lung cells were co-cultured with Peripheral Blood Mononuclear Cells (PBMC), which were then exposed to PM10 for 24 hrs. Cytokines profile was determined using a multiplex magnetic bead assay using a dual laser flow analyzer Luminex 200. Our results show that Co-Cultured cells highly amplified the immune response when compared to single cell analyzes. PM exposure particularly from Urban Samples (Guaynabo Site), were significantly induced as determined by interleukin 6, 8 and 10 concentration. Single cell exposure to PM had showed no effect on IL10 released but was significantly induced during co-cultured conditions.

1906 Inhalation of Fine Particles from Mexico City Increases Exacerbations in an Asthma Model

C. I. Falcon-Rodriguez[2,5], A. Osornio-Vargas[4], A. De Vizcaya-Ruiz[3], I. Rosas-Perez[1] and P. Segura-Medina[2]. *[1]Departamento de Aerobiologia, Centro de Ciencias de la Atmósfera, Mexico City, Mexico; [2]Departamento de Investigacion en Hiperreactividad bronquial, INER, Mexico City, Mexico; [3]Departamento de Toxicologia, CINVESTAV, Mexico City, Mexico; [4]Department of Pediatrics, University of Alberta, Edmonton, AB, Canada and [5]Posgrado en Ciencias Biologicas, UNAM, Mexico City, Mexico.* Sponsor: A. de Vizcaya-Ruiz.

Air pollution is a big problem around the world, especially in megacities like Mexico City, where contamination increases day to day. Several pollutants may contribute to asthma development, which is a chronic airway disease characterized by airway inflammation and reversible obstruction. The correlation between asthma and exposure to Particulate matter (PM) is controversial. So our aim was to determine whether inhalation of fine particles from Mexico City can increase the asthmatic exacerbations in a guinea pig model. Animals were sensitized to ovalbumin (OVA)+AlOH3 and exposed to filtered air (FA) or PM2.5 (daily 5h/3days), using an Aerosol Concentrator System (North of Mexico City, CINVESTAV-Zacatenco). We evaluated lung function by plethysmography to obtain an airway resistance index (Penh) after OVA challenge. Animals were euthanized, and Broncho-alveolar Lavage (BAL) was performed to determine the percentage different cellular elements by Giemsa-stained, and we did ELISA assay for asthma (OVAIgG1) and an epithelial damage biomarker (CC16). Lung samples were recovered for histology and PAS-stained. Our results showed exposure to PM2.5 increases Penh, arose asthma and damage biomarkers, such as OVA-IgG1 and CC16 levels in BAL. Also, in asthmatic exposed to FA increased eosinophils. However, PM2.5 arose also neutrophils. As well as arising mucous production in the bronchiolar epithelium, where lack mucous cells. Our results suggest that inhalation of fine particles from Mexico City in a guinea pig asthma model can increase obstruction and exacerbations. However, we could associate the neutrophilia with fatal bronchospasm as a forecast of fatal asthma. The author is a Scholarship holder CONACYT: 233789. The study was supported by SECITI 042/2013.

1907 The Effects of TNFα on Gap Junctions in Lung Epithelial Cells Exposed to Polycyclic Aromatic Hydrocarbons

D. Romo[2], R. S. Osgood[2], K.-N. Xiong[2], J. Xiong[2] and A. K. Bauer[2]. *[1]Environmental and Occupational Health, University of Colorado Denver, Aurora, CO and [2]Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Pulmonary diseases, such as chronic obstructive pulmonary disease (COPD), are linked to cigarette smoke exposure. We study second- and third-hand smoke abundant low molecular weight polycyclic aromatic hydrocarbons (LMW PAHs). We previously demonstrated that two of these LMW PAHs, 1-methylanthracene (1-MeA) and fluoranthene (Flthn), inhibit gap junctions, decrease connexin 43 (Cx43) expression, activate MAP kinases, and induce inflammatory mediators, such as tumor necrosis factor alpha (TNF). Gap junctional intercellular communication (GJIC) is involved in lung tissue homeostasis and GJIC inhibition could potentiate pre-existing disease states. TNF is a pro-inflammatory cytokine used as a biomarker for COPD. In this study, we hypothesized that TNF inhibits GJIC alone and that a LMW PAH mixture (1-MeA and Flthn) exacerbates this response. We used MTS assays for cytotoxicity, scapel-loaded dye transfer assays to measure GJIC, connexin (Cx)43 immunoblots, as well as TNF antibody neutralization in a mouse alveolar type II pneumocyte cell line (C10 cells) in the absence or presence of a 1-MeA and Flthn mixture at two time points. Cytotoxicity was not observed with any treatment up to 48 h. After 4 h, TNF inhibited GJIC significantly

at all doses (0.1 -20 ng/ml) compared to control and at 24 h the inhibition was further enhanced. The PAH mixture in combination with TNF treatment did not further reduce GJIC after 4 h. However, after 24 h we observed a significant GJIC reduction with combined TNF and PAH mixture exposure when compared to single compound treatment. Cx43 immunoblots and TNF neutralization both corresponded to the GJIC findings. These results support a role of TNF on lung epithelial cell GJIC dysregulation and suggest that individuals with pre-existing lung disease may be more susceptible to environmental exposures when compared to healthy controls. Future studies will validate these results using human cell lines and co-culture studies.

PS 1908 Cigarette Smoke Mediated Regulation of Epigenetic Signatures on NF-κB Proximal Promoter Region in Murine Macrophages and Human Monocytes

G. Kaur[2], A. Panday[1], A. Grove[1] and S. Batra[2]. [1]*Biochemistry, Louisiana State University, Baton Rouge, LA and* [2]*Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.* Sponsor: S. Batra.

Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death in the United States. Active or passive cigarette smoke (CS) exposure is attributed to be its principal cause. Transcription factor NF-κB plays a key role in regulating inflammatory responses in COPD patients. Our initial studies using murine macrophages and THP-1 cells showed CS-mediated increase in the expression of NF-κB and therefore we hypothesized that NF-κB expression can be under epigenetic control. We tested two epigenetic signatures on histone H3 at NF-κB proximal promoter region (PPR). These included acetylation of lysine-9 (H3K9ac) and trimethylation of lysine-4 of Histone 3 (H3K4me3). We also investigated the effect of histone deacetylase (HDAC) inhibitor benzyl Isothiocyanate (BITC) on H3K9ac at NF-κB PPR. For these studies. RAW 264.7 murine macrophages and THP-1 cells were exposed to CS or DMSO (control) for 24 hours. Following treatment/challenge, the cells were harvested and fixed using formaldehyde. Chromatin immunoprecipitation was performed using anti-H3K9ac and anti-H3K4me3 and samples were quantified using Real-Time PCR. We observed a significant decrease in H3K4me3 and an increase in H3K9ac at NF-κB PPR following CS exposure in both RAW 264.7 macrophages and THP-1 cells. Although we anticipated BITC-mediated increase in acetylation at lysine 9 of Histone 3 at NF- κB PPR, we found BITC-mediated downregulation of H3K9ac at NF-κB PPR in CS-challenged murine macrophages. Currently we are investigating the effect of BITC treatment on global histone 3 expression and acetylation in CS challenged cells.

PS 1909 Salicylates Improve Hyperoxia-Compromised Phagocytic Function in Macrophages Through Inhibition of HMGB1 Release

M. Wang[2] and L. L. Mantell[1,2]. [1]*Feinstein Institute for Medical Research, North Shore-LIJ Health System, Manhasset, NY and* [2]*Department of Pharmaceutical Science, St John's University, Queens, NY.*

High Mobility Group Box 1 (HMGB1) is an important chromatin protein that facilitates DNA folding and transcription in the nucleus and acts as a proinflammatory cytokine when it is released to the extracellular milieu. Prolonged exposure of mice to hyperoxia contributes to the release of HMGB1 into the airways from lung cells, subsequently affecting their bacterial clearance in the lungs. We have recently shown that hyperoxia-induced NF-κB activation plays a critical role in mediating the release HMGB1 from macrophages. Salicylates, the well-known analgesic and anti-inflammatory agents, exhibit inhibitory effects on NF-κB activation in cancer cell lines. The present study tested the hypothesis that salicylates can improve hyperoxia-compromised phagocytic functions in macrophages through inhibiting the release of HMGB1 from macrophages via its effects on hyperoxia-induced NF-κB activation. Cytotoxicity of salicylates was first determined in both primary and transformed cultured macrophages by treating macrophage-like RAW 264.7 cells and bone marrow derived macrophages (BMDMs) with salicylates at doses up to 2mM under hyperoxic conditions. We show here that salicylates at doses similar to those used clinically (≤ 200uM), did not induce detectable cytotoxicity. On the other hand, salicylates at these doses decreased hyperoxia-induced accumulation of HMGB1 in the cell culture media. Importantly, treatment of both RAW 264.7 cells and BMDMs enhanced their phagocytic activities that were compromised by prolonged exposure to hyperoxia. These results indicate the potential of salicylates in the enhancement of phagocytosis of alveolar macrophages under hyperoxic condition via inhibiting the release of HMGB1. These preclinical findings could be translated into clinical settings to improve the current treatment of ventilator associated pneumonia and other HMGB1 mediated diseases.

PS 1910 Regulation of Macrophage Accumulation and Activation in the Lung Following Ozone Exposure by Farnesoid X Receptor

M. Francis, G. Guo, R. Sun, J. Cervelli, H. Choi, B. Kong, J. D. Laskin and D. L. Laskin. *Rutgers University, Piscataway, NJ.*

Ozone is a ubiquitous urban air pollutant known to damage the lung. Evidence suggests that inflammatory macrophages contribute to ozone toxicity. Farnesoid x receptor (FXR) is a nuclear receptor and a bile acid sensor reported to negatively regulate the transcription factor, NFκB and consequently, down regulate macrophage inflammatory responses. In these studies, we analyzed the role of FXR in ozone-induced lung inflammation and macrophage activation. Treatment of C57BL/6 (WT) mice with ozone (0.8 ppm, 3 h) resulted in increased FXR mRNA and protein expression in the lung. This was associated with a marked increase in numbers of proinflammatory Ly6CHi macrophages in the lung and upregulation of macrophage inducible nitric oxide synthase (iNOS) and toll-like receptor (TLR)4 expression. Loss of FXR resulted in greater numbers of Ly6CHi macrophages in the lung and prolonged upregulation of iNOS and TLR4, indicating chronic inflammation and macrophage activation. Loss of FXR also resulted in increased numbers of circulating Ly6CHi proinflammatory blood monocytes. Conversely, numbers of YM1+ anti-inflammatory macrophages were decreased in lungs of FXR-/- mice after ozone exposure, while numbers of anti-inflammatory Ly6CLo macrophages were increased. These data are consistent with idea that there are multiple subsets of anti-inflammatory macrophages responding to ozone and that pathways regulating their activity are distinct. Changes in lung macrophage populations in FXR-/- mice were linked to exacerbation of ozone toxicity, as measured by increased expression of cytochrome b5, a marker of mitochondrial dysfunction and oxidative stress; ozone-induced structural alterations in the lung were also more prominent in FXR-/- mice, characterized by bronchiolization of the alveolar septal wall and interstitial thickening. These responses were independent of effects on liver function, as measured by serum transaminases and bile acid concentrations. These data suggest that FXR plays a role in limiting lung inflammatory responses to ozone. Supported by NIH Grants ES004738, AR055073, ES007148 and ES005022.

PS 1911 Resolvin D1 Dampens Pulmonary Inflammation and Promotes Nontypeable *Haemophilus influenzae* Clearance

A. Croasdell[2], S. Lacy[2], T. H. Thatcher[2], P. J. Sime[2] and R. P. Phipps[2]. [1]*Department of Environmental Medicine, University of Rochester, Rochester, NY and* [2]*Department of Medicine, University of Rochester, Rochester, NY.*

Many toxic stimuli induce excessive pulmonary inflammation, promoting a chronic inflammatory environment that leads to tissue destruction, disease, and a disruption of normal host-pathogen interactions resulting in increased viral and bacterial exacerbations. Nontypeable *Haemophilus influenzae* (NTHi) is a gram-negative, opportunistic pathogen which causes exacerbations and contributes to persistent and excessive inflammation, indicating a failure to resolve, even after the infection is cleared. Recent investigations have led to the discovery of endogenously produced, specialized pro-resolving mediators (SPMs). These lipid mediators play a critical role in the active resolution of inflammation by both dampening pro-inflammatory actions and promoting resolution pathways. Importantly, resolvins are not immunosuppressive. Based on these characteristics, we hypothesized that 17(R)-Resolvin D1 (RvD1) would dampen NTHi-induced inflammation while still enhancing bacterial clearance. C57Bl/6 mice were treated with RvD1 and infected with live NTHi. RvD1 increased macrophage numbers while decreasing neutrophil influx following infection, and temporally regulated pro-inflammatory cytokines. Importantly, RvD1 treated mice had fewer NTHi colony forming units, with this clearance mediated by enhanced phagocytosis from M2 alternative macrophages. Finally, RvD1 improved the overall respiratory health of the mice, with reductions in weight loss, hypothermia, respiratory rate, and loss of O2 saturation. This research is a first step in investigating the role of SPMs in pulmonary bacterial infections, and will provide the groundwork for further investigation into SPMs as alternatives to immunosuppressive therapies like steroids. Supported by NIEHS T32ES007026, NIH RO1HL120908, CTSI Incubator (NIH UL1RR024160), CTSI 8UL1TR000042 and the PhRMA Foundation.

1912 CF Airway Epithelia Contribute to the Compromised Innate Immunity Against Bacterial Infection

X. Yang[3], V. Patel[3], M. Wang[3], D. D. Thomas[2] and L. L. Mantell[1,3]. [1]*Center for Inflammation and Immunology, The Feinstein Institute for Medical Research, North Shore-LIJ Health Science, Manhasset, NY;* [2]*Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL and* [3]*Department of Pharmaceutical Sciences, St. John's University, Jamaica, NY.*

Chronic bacterial infections and neutrophilic inflammation are characteristics of the airways in patients with CF. *Pseudomonas aeruginosa* (PA) remains one of the most prevalent bacterial pathogens affecting majority of adult CF patients. Previously, we demonstrated that the elevated levels of HMGB1 in the airways of CF patients and cystic fibrosis transmembrane conductance regulator (CFTR)-/- mice, play a pivotal role in the pronounced inflammatory responses and the impairment of PA clearance from CF lungs. The objective of this study was to investigate the role of airway epithelia in the accumulation of HMGB1 in the CF airways, using an *in vitro* model of CF airway epithelial IB3-1 cells and CFTR corrected S9 cells. Here, we report that IB3-1 cells release more HMGB1 into the extracellular milieu than S9 cells at basal levels. In addition, treatment of IB3-1 cells with PA LPS (0.1, 1 and 10 µg/ml) for 24 hrs resulted in a further increase in the accumulation of HMGB1 in the cell culture media. Next, we sought to determine whether ethyl pyruvate, a well-known inhibitor of HMGB1 release from macrophages, affects the baseline release of HMGB1 from CF epithelia. IB3-1 cells were treated with different concentrations of ethyl pyruvate (0, 0.01, 0.1, 1, 10 and 20mM) for 24h. Interestingly, levels of HMGB1 accumulation were not significantly reduced by ethyl pyruvate, indicating that ethyl pyruvate does not affect baseline HMGB1 release from CF epithelial cells. These results suggest that baseline HMGB1 release from CF epithelia is via an alternate pathway that is different from that in macrophages. The production of nitric oxide (NO), an important immune response is essential in the clearance of bacterial infections. Interestingly, IB3-1 cells had reduced levels of NO production upon exposure to heat-killed PA. In conclusion, we show that CF airway epithelial cells play an important role in contributing to the elevated airway levels of HMGB1 and reduced production of NO, suggesting that CF epithelia contribute to the persistent PA infections in CF patients.

1913 DHA Suppresses Macrophage Inflammatory Responses by Attenuating Notch Signaling Pathways

L. K. Rogers[1,2], K. M. Heyob[1] and M. Ali[1]. [1]*Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH and* [2]*Pediatrics, The Ohio State University, Columbus, OH.*

Backgound. Persistent macrophages were observed in the lungs of murine offspring exposed to maternal LPS and neonatal hyperoxia. Maternal docosahexaenoic acid (DHA) supplementation prevented the accumulation of macrophages and improved lung development. We hypothesized that these macrophages are responsible for the pathologies observed in this model and the effects of DHA supplementation. Methods. Primary macrophages were isolated from adult mice fed control diets or DHA supplemented diets. Macrophages were exposed to hyperoxia (O2) for 24 h and LPS for 6 h or 24 h. Additional lung macrophages isolated from mice fed standard chow were treated with DHA *in vitro* and exposed to hyperoxia (O2) for 24 h and LPS for 6 h or 24 h. Expression of NOTCH pathway proteins, caspase 9, and HMGB1 release were assessed by western blot. Treated primary macrophages were co-cultured with MLE12 cells. HMGB1 and LTB4 were measured in the media and Ki-67 (proliferation) and cl-caspase 3 (apoptosis) were assessed in MLE12 cells by flow cytometry. Results. Significant attenuation of Notch 1 and Jagged 1 protein levels were observed in response to DHA supplementation *in vivo* but similar results were not evident in macrophages isolated from mice fed standard chow and supplemented with DHA *in vitro*. Co-culture of activated macrophages with MLE12 epithelial cells resulted in the release of HMGB1 and LTB4 from the epithelial cells and this release was attenuated in the cultures containing macrophages previously supplemented with DHA. Furthermore, proliferation was preserved and apoptosis was decreased in MLE12 cells co-cultured with macrophages supplemented with DHA compared to controls. Conclusion. Collectively, our data indicate that long term supplementation with DHA as observed *in vivo*, resulted in suppression of Notch 1/Jagged 1 protein expression. However, DHA supplementation both *in vivo* and *in vitro* was sufficient to suppress release of HMGB1 or LTB4 and to protect epithelial cells in co-culture.

1914 Bisphenol A and Phthalate May Promote Endometriosis via affecting Inflammatory Phenotype of Peritoneal Macrophages

Q. Li[2], M. K. Bagchi[2] and I. C. Bagchi[2]. [1]*Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL and* [2]*Molecular & Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL.*

Endometriosis is defined as growth of endometrial tissues outside the uterus and characterized as an inflammatory disease due to an imbalance in inflammatory and anti-inflammatory signals. Ample evidence suggests that macrophage (Mφ) is a key component of endometriosis. Our recent studies showed that in the presence of endometrial cells, peritoneal Mφ underwent a transition from pro-inflammatory (Mφ1) to anti-inflammatory (Mφ2) subtypes. Infiltration of both types of Mφ was observed in ectopic lesions. Early studies showed that Mφ is vulnerable to exposure of immunotoxicants, such as Bisphenol A (BPA) and phthalate (DEHP), two plastic chemicals widely used in our daily life. Although it is known that BPA and phthalate adversely affect macrophage functions, a comprehensive study of the exposure of low level of BPA or DEHP on pro-inflammatory or anti-inflammatory function of Mφ is still lacking. In this study, peritoneal naïve Mφ were exuded and exposed to BPA or DEHP-metabolite, MEHP, at a range from 2 to 2000 ng/ml for 24 hours. These cells were then activated by suboptimal doses of LPS plus IFNγ, with/without IL-10 (Mφ1), or IL-4 (Mφ2) for 12 hours to evaluate mRNA expression and cytokine production. Our results showed that low doses of BPA or MEHP (2 or 20 ng/ml) enhanced Mφ1-derived pro-inflammatory cytokines, e.g., IL1b, IL6, CCL2, CXCL10, and TNFα, while higher doses of these compounds (200 or 2000 ng/ml) exhibited minimal or inhibitory effects. In addition, there was an overall decline in NOS2 expression, NO production, and M2 marker expression, e.g., Ym1, mannose receptor, and Fizz1 in response to BPA, while MEHP showed negligible effects on M2 markers. Neither BPA nor MEHP exhibited any effect on IL-10-mediated suppression of pro-inflammatory cytokines. These results indicate that BPA and MEHP at human exposure levels may affect the pro-inflammation and anti-inflammation balance of macrophages, which is potentially important in the pathogenesis of endometriosis.

1915 Role of Lipids in Macrophage Foam Cell Formation During Nitrogen Mustard-Induced Lung Fibrosis

A. Venosa, A. J. Gow, J. D. Laskin and D. L. Laskin. *Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

Nitrogen mustard (NM) is a cytotoxic vesicant known to target the lung, causing acute injury which progresses to fibrosis. Evidence suggests that distinct proinflammatory/cytotoxic and profibrotic macrophage subsets contribute to these lung pathologies. In these studies, we assessed whether NM-induced alterations in lung lipids contributes to the development of profibrotic macrophages and fibrosis. Treatment of rats with NM (0.125mg/kg, i.t.) resulted in a persistent increase in bronchoalveolar lavage fluid phospholipid content, suggesting altered lipid homeostasis. A time-related increase in oxidized lipids was also observed in the lung. This was correlated with upregulation of macrophage scavenger receptors (CD36, CD68 and CD163), which are important in uptake of oxidized lipids, and down regulation of cholesterol efflux transporters ABCA1 and ABCG1. This was evident beginning 3 d post NM and continuing for at least 28 d, a time coordinated with the development of lung fibrosis. At 28 d, lung macrophages were enlarged and vacuolated, and localized in fibrotic regions of the lung; macrophages also stained positively for Oil-red-O, a prototypical marker of foam cells. Lipid accumulation in macrophages after NM exposure was associated with decreased expression of liver-X-receptor (LXR), a nuclear receptor that down regulates scavenger receptors and cholesterol efflux transporters. These data suggest that NM induces alterations in lipid metabolism resulting in lipid accumulation in macrophages and the formation of foam cells, which we speculate contribute to lung fibrosis. Supported by NIH grants AR055073, ES004738, HL096426 and ES005022.

1916 Ascorbic Acid Attenuates Hyperoxia-Compromised Host Defense Against Pulmonary Bacterial Infection

V. S. Patel[4], V. Sampat[4], M. G. Espey[1], R. Sitapara[4], H. Wang[2], X. Yang[4], R. Kundu[4], C. R. Ashby[4], D. D. Thomas[3] and L. L. Mantell[2,4]. *[1]National Cancer Institute, Bethesda, MD; [2]The Feinstein Institute for Medical Research, North Shore-LIJ Health Science, Manhasset, NY; [3]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL and [4]Pharmaceutical Sciences, St. John's University, Jamaica, NY.*

Supraphysiological concentrations of oxygen (hyperoxia) can compromise host defense and increase susceptibility to bacterial infections, causing ventilator-associated pneumonia (VAP). The phagocytic activity of macrophages is impaired by hyperoxia-induced increases in the levels of reactive oxygen species (ROS) and extracellular high mobility group box protein B1 (HMGB1). Ascorbic acid (AA), an essential nutrient and antioxidant, has been shown to be beneficial in various animal models of ROS-mediated diseases. The aim of this study was to determine whether AA could attenuate hyperoxia-compromised host defense and improve macrophage functions against bacterial infections. Male C57BL/6 mice were exposed to hyperoxia (≥98% O2, 48 h), followed by intratracheal inoculation with *Pseudomonas aeruginosa*, and simultaneous intraperitoneal administration of AA. AA (50 mg/kg) significantly improved bacterial clearance in the lungs and airways, and significantly reduced the accumulation of HMGB1 in the airways. The incubation of RAW 264.7 cells (a macrophage like cell line) with AA (0-1000 μM) prior to hyperoxic exposure (95% O2) stabilized the phagocytic activity of macrophages in a concentration-dependent manner. The AA-enhanced macrophage function was associated with significantly decreased production of intracellular ROS and accumulation of extracellular HMGB1. These data suggest that AA supplementation can prevent or attenuate the development of VAP in patients receiving oxygen support.

1917 Novel Para-Phenyl Substituted Diindolylmethanes Inhibits Inflammatory Response in Murine Synovial Fibroblasts

M. F. Afzali[2], S. H. Safe[1], W. H. Hanneman[2] and M. E. Legare[2]. *[1]Center for Environmental and Genetic Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX and Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX and [2]Center for Environmental Medicine, Colorado State University, Fort Collins, CO.*

Rheumatoid Arthritis (RA) is a chronic inflammatory disease, primarily affecting the joints. Disease progression involves the thickening of the synovial lining due to the proliferation of fibroblasts- like synoviocytes and infiltration by inflammatory cells. This proliferative mass, or pannus, invades and destroys articular cartilage and bone. The involvement of resident, fibroblast- like cells contributes significantly to the perpetuation of disease and even playing a role in its initiation. The established model concerning cytokine network in RA is that tumor necrosis factor- alpha (TNF-α) is a critical effector in the pro-inflammatory cytokine cascade. New treatment modalities in RA have applied systemic cytokine blockade. A series of novel, para-substituted diindolylmethane compounds (C-DIMs) have shown to modify selective inflammatory pathways in pancreatic, bladder, and colon cancer studies, as well as models of neuroinflammation. In this study, we postulated that C-DIM12 will have anti-inflammatory effect in primary synovial fibroblasts, the suppression of which could lead to an alternative cell target for RA treatment. In this investigation primary synovial fibroblasts were isolated from murine ankle joints and characterized using immunofluorescence and flow cytometry. Immunofluorescence confirmed positive Vimentin expression. Flow cytometric analysis of primary culture revealed > 80-90% CD90.2 positive, < 1% Mac-1 positive, > 85% (VCAM-1) positive and > 80% (ICAM-1). TNF- α stimulated synovial fibroblasts were used to elucidate underlying mechanisms of action. Vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1) are constitutively expression on synovial fibroblasts and surface expression is further increased after exposure to TNF-α. C-DIM12 significantly suppressed the expression of VCAM-1 and cytokines. These results indicate that C-DIM12 in this model of RA can be useful as an alternative therapeutic for treatment of RA and other arthritic conditions.

1918 Blocking Immune Tolerance Unmasks the Potential for a Drug to Cause Idiosyncratic Liver Injury

A. Mak and J. Uetrecht. *University of Toronto, Toronto, ON, Canada.*

Our laboratory recently reported what we believe is the first valid animal model of IDILI by treating PD1-/- mice with an anti-CTLA-4 antibody and amodiaquine (AQ). PD1 and CTLA-4 are important immune checkpoint receptors that are involved in inducing immune tolerance. This model was able to produce significant liver injury that looks very similar to the liver injury seen in humans. Although this model was shown to work with AQ, the question becomes whether blocking immune tolerance will unmask the potential of other drugs to cause IDILI and if this model can distinguish between safe and dangerous drugs. In this study we tested isoniazid and nevirapine, both drugs with significant history of causing IDILI in humans. Both drugs in combination with these immune checkpoint inhibitors caused mild but significant delayed onset liver injury, which is similar to the mild injury that they can cause in humans. This animal model was further tested in this study by using this combination of immune checkpoint inhibitors along with troglitazone, and pioglitazone. Troglitazone has a history of causing IDILI in humans, while pioglitazone does not. PD1-/- mice treated with anti-CTLA-4 and troglitazone showed a significant delayed onset rise in ALT levels, while the same combination of immune checkpoint inhibitors and pioglitazone showed no injury. These results suggest that blocking immune tolerance may be a general method to unmask the potential of drugs to cause IDILI and also provides promising evidence that this model can be used to screen drugs for their potential to cause IDILI. Supported by grants from the Canadian Institutes for Health Research.

1919 Metformin Effect on Regulation of CXCL8 Production

Z. Xiao, W. Wu, I. Ban and V. Poltoratsky. *Pharmaceutical Sciences, St. Johns University, Jamaica, NY.*

Interleukin (IL)-8, also known as CXCL8, belongs to pro-inflammatory cytokines and CXC (cysteine-X-cysteine) amino acid chemokine family. In the normal immune responses, its up-regulation leads to inflammatory reactions, and ultimately results in pathogen elimination. As a chemokine, CXCL8 is capable of recruiting immune cells, such as macrophages, neutrophils, and lymphocytes, further enhancing scavenge of pathogens. However, in the cases of malignancies as breast cancer, CXCL8 acts in a autocrine and/or paracrine manner functioning as growth factor that promote tumor cells proliferation. As a chemokine, it also recruits immune cells which may exert roles in promoting tumor progression. Furthermore, clinical studies indicate that patients who have elevated CXCL8 levels are associated with poor prognosis. Investigation of the role of CXCL8 in tumor progression and its regulatory mechanism may shot light on new targets, however may work adjunctively to the conventional treatments, for anti-cancer therapies. This study is intended to address the role of metformin, the drug conventionally used in type II diabetes, in inhibition of CXCL8 synthesis and release. By using ELISA, we tested the elevated CXCL8 concentration, induced by LPS, in cell culture supernatant. Metformin pre-treated (24h) groups shows inhibition on CXCL8 release in a dose-dependent fashion. In line with the previous data, qPCR results show decreased CXCL8 expression levels in metformin pre-treated groups with a significant suppression in 10mM metofrmin groups. To investigate the regulatory mechanism of CXCL8 expression, immunofluorescence results reveal translocation of the p65, one of the subunit of transcriptional factor NF-κB, induced by LPS treatment indicating the role of NF-κB pathway in regulating CXCL8 expression. The suppression of translocation activities caused by metformin pre-treatment suggest its role in inhibiting the activity of transcriptional factor NF-κB. Luciferase assay results further validate the dampened transcriptional activities of NF-κB brought by metformin treatment. Altogether, our data suggest metformin dampens the transcriptional activities of NF-κB that are essential in chemokine CXCL8 expression.

1920 Trpv4 Modulates Cyp2e1-Mediated Oxidative Stress Toxicity and Kupffer Cell Activation in Nonalcoholic Steatohepatitis

R. K. Seth[2], S. Das[2], D. Dattaroy[2], F. Alhasson[2], G. A. Michelotti[1], M. Nagarkatti[6], P. Nagarkatti[6], P. D. Bell[4], W. B. Liedtke[3], A. M. Diehl[3] and S. Chatterjee[2]. *[1]Metabolon, Inc., Durham, NC; [2]ENHS, University of South Carolina, Columbia, SC; [3]Gastroenterology, Duke University, Durham, NC; [4]Medicine, University of Alabama, Birmingham, AL; [5]Neurology, Duke University, Durham, NC and [6]PMI, University of South Carolina SOM, Columbia, SC.*

Emerging evidence shows that oxidative stress via the activation of cytochrome p450 2E1 (CYP2E1) is key to progression of inflammation in nonalcoholic steatohepatitis (NASH). We have shown previously that CYP2E1 mediated oxidative stress and macrophage polarization in NASH was attenuated by NO donor. However the molecular mediators and its pathways that regulate CYP2E1 mediated oxidative stress in NASH remains obscure. For this study we used a high fat diet induced obese mice as *in vivo* and HepaRG, Kupffer cells as *in vitro* model. The CYP2E1 substrate pyrazole or BDCM were used to induce CYP2E1 mediated oxidative stress, inflammation and NASH pathology. Results showed that the transient receptor potential vanilloid channel 4 (TRPV4) expression and protein levels were significantly elevated in parallel to increases in CYP2E1 and correlated well with increased lipid peroxidation, IL1β, MCP1, TNFα and HMGB1 levels in the NASH livers and in Kupffer cells. TRPV4 knockout (KO) mice showed increased CYP2E1 protein, lipid peroxidation, inflammatory cytokines, infiltration of leukocytes, sinusoidal endothelial dysfunction (SED) maker genes (CD34, cdh5, ICAM-1 and VEGFR2), HMGB1 levels, decreased phosphorylated endothelial nitric oxide synthase (NOS3) and exhibited early morbidity as compared to wildtype mice with NASH. Mechanistically, diallyl sulfide (CYP2E1 inhibitor) or NO donor DETANONOate administration to TRPV4 KO mice completely abrogated enhanced NASH symptoms and morbidity. Interestingly, use of NO donor significantly decreased CYP2E1-mediated lipid peroxidation, an indirect measure of its activity, proinflammatory genes and SED marker in TRPV4 KO mice. The results obtained show that TRPV4, a crucial protein responsible for sensing changes in osmotic pressure and Ca2+ also regulates CYP2E1-mediated oxidative stress, inflammation and endothelial injury probably by activating NOS3 and release of nitric oxide. Based on the above, targeting TRPV4 or its downstream signaling cascade might be a promising therapeutic strategy in NASH.

1921 Comparison of Early vs Late Pulmonary Toxicity in Crystalline Silica Exposed Rats

P. Joseph, J. Roberts, T.-h. Chen, W. McKinney, M. Orandle and C. Umbright. *NIOSH, Morgantown, WV.*

Occupational exposure to respirable crystalline silica can result in silicosis in addition to other adverse health effects. Currently, we investigated and compared the early vs. late pulmonary toxicity induced by inhalation exposure of rats to crystalline silica. In addition, differential expression of specific genes involved in known mechanisms of silicosis viz. inflammation and fibrosis, were determined in the principal target organ of silica toxicity (lung) and a surrogate tissue (blood) in the rats. Rats were exposed by inhalation to air (control) or respirable crystalline silica (Min-U-Sil 5 Silica) at a concentration of 15 mg/m3, for 6 hours per day for 5 days. The rats, following exposure, were maintained under standard animal housing conditions either for 1- or 9-months and euthanized. Silica-induced pulmonary toxicity was determined on the basis of lung histology, and bronchoalveolar lavage (BAL) parameters of toxicity (lactate dehydrogenase activity, number of alveolar macrophages and polymorphonuclear leukocytes, and generation of reactive oxygen species). Differential expressions of specific genes involved in inflammation and fibrosis were determined in the lungs and blood using PCR arrays. Mild inflammation was the only histological change detected in the rat lungs at the 1-month post-exposure period whereas type II pneumocyte hyperplasia and fibrosis were detected in the lungs at the 9-months post-exposure period. Similarly, compared to the early time period, more significant changes in all BAL parameters of toxicity were noticed in the rats at the late post-exposure period. Differential expression of several genes associated with inflammatory response and fibrosis were detected in the lungs and blood of all of the silica exposed rats. However, both the number of significantly differentially expressed genes and the changes in gene expression were greater at the 9-month post-exposure period compared with the 1-month period. Collectively, these results, demonstrated the critical role of post-exposure time interval in the progression of silica-induced pulmonary toxicity in rats.

1922 Understanding Spatiotemporal Signaling Associated with Inflammation Caused by a Physical Stressor

J. Boyd[1], A. Han[1], E. Fabyanic[1], M. Prediger[1] and H. Currie[2]. *[1]Bennett Department of Chemistry, West Virginia University, Morgantown, WV and [2]Department of Chemistry and Physics, California University of Pennsylvania, California, PA.*

While temporal aspects of inflammation have been extensively studied and provide an important understanding of the processes involved in both toxicity and repair, research into the disparate spatial response to localized inflammation is sparse. This study addresses spatial and temporal differences of phosphoproteins found in muscle tissue following a traumatic femur fracture in Sprague-Dawley rats, which are further compared to co-localized cytokine responses. In particular, several proteins (AKT, ERK, c-Jun, CREB, JNK, MEK1, and p38) associated with inflammation, new tissue formation, and remodeling were found to exhibit significant spatial and temporal differences in response to localized traumatic injury. In addition, post-translational phosphorylation levels were measured to further capture the contribution of protein activity during the recovery phase. Our results identified generally lower degrees of phosphorylation at the site of injury (compared to sites located further away) at early time points (beginning immediately following fracture through 24 hours). Further, an increase of phosphorylation for select proteins (at or near the injury site) was observed at the last time point measured in this study (168 hours). Finally, phosphoprotein measurements were found to be significantly correlated to cytokine responses (IL-1α, IL-1ß, IL2, IL6, TNF-α, and MIP-1α), suggesting the importance of coordinated intracellular and extracellular activity during crucial periods of inflammation and repair. This study represents a first attempt to monitor coordinated changes in extracellular and intracellular signaling related to traumatic injury in muscle tissues, which may provide a framework for future research to improve our understanding of the spatiotemporal response inflammation.

1923 Suppresion of Gastric Inflammatatory Markers and Mitochondrial Apoptotic Pathway by Methanol Extract of *Chasmanthera dependens* Stem in Ethanol-Induced Gastric Ulcer Healing

A. S. Tijani, O. E. Farombi and S. B. Olaleye. *Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria; Gastrointestinal Research Unit, Department of Physiology, University of Ibadan, Ibadan, Nigeria.*

Neutrophil infiltrations and continuous generation of reactive oxygen species have been reported to delay gastric ulcer healing in ethanol-induced gastric ulcer. This study investigated the potential of the methanol extract of *Chasmanthera dependens* (MECD) stem in healing ethanol-induced gastric ulcer in male Wistar rats. Thirty six rats were divided into six groups of six rats each and treated orally. Group 1 rats served as control group and received 1 ml/kg body weight of 1% gum acacia solution; groups 2-6 rats were given acidified ethanol to induce gastric ulcer. Groups 3, 4 and 5 rats were treated with 200, 400 and 800 mg/kg body weight of MECD stem while group 6 rats were treated with 50 mg/kg body weight of cimetidine (CIM) for fourteen days after ulcer induction. Ulcer score, ulcer index and levels of tumor necrosis-alpha (TNF-α), interleukin-1beta (IL-1β), cytochrome c (Cyt-c), caspase-9 (Casp-9) and caspase-3 (Casp-3) were assessed in the serum and gastric tissues were used for histological examination and terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL) assay. Acidified ethanol caused severe gastric mucosa damage with ulcer score and ulcer index of 19.00 ± 1.00 and 2.86 ± 0.37 at $p<0.01$ respectively. But treatments with MECD significantly heal the ulcer with percentage ulcer healing of 78.36 ± 0.62, 92.36 ± 1.86, 81.41 ± 0.25 for different doses of MECD and CIM, 72.12 ± 0.30 respectively. Similarly, ethanol administration increased TNF-α, IL-1β, Cyt-c, Casp-9, Casp-3 levels and number of positive apoptotic nuclei in ulcerated untreated group, while MECD or CIM treatment for 14-days significantly reversed these observations and the histological examination revealed restitution of the gastric tissues. The results show that administration of MECD stem promotes gastric ulcer healing by suppressing the inflammatory markers and inhibiting the intrinsic apoptotic pathway and as such could be relevant in pathologies involving gastrointestinal dysfunction such as ulcer.

1924 Classical and Alternative Activation of Cyanobacteria *Scytonema javanicum* and *Scytonema ocellatum* Lipopolysaccharide-Treated Rat Brain Microglia

L. C. Klemm[2], M. L. Hall[2], P. Williams[1] and A. M. Mayer[2]. [1]*Chemistry and Biochemistry, University of Hawaii at Manoa, Honolulu, HI and* [2]*Pharmacology, Midwestern University, Downers Grove, IL.*

Background: Cosmopolitan Gram-negative cyanobacteria may contaminate freshwater by releasing toxins, such as lipopolysaccharide (LPS), thus affecting human and animal health. We recently reported that cyanobacterium *Oscillatoria* sp. LPS elicited classical and alternative activation of rat microglia (BMG) *in vitro* (The Toxicologist, 144: Abstract 137, 2015). We hypothesized that both cyanobacteria *Scytonema javanicum* (Scyj) and *Scytonema ocellatum* (Scyo) LPS would activate classical and alternative activation of BMG *in vitro* and concomitant release of cytokines and chemokines. Methods: Scyj LPS and Scyo LPS were prepared by hot phenol/water extraction. *E. coli* LPS (Ec LPS) 026:B6 from Difco Lab, Detroit, MI was used as a positive control. BMG were isolated from neonatal rats, and treated *in vitro* with either Scyj LPS, Scyo LPS, or Ec LPS in a concentration-dependent manner for 18 hours at 35.9° C. Cytokines and chemokines were determined using a Milliplex® MAP rat cytokine/chemokine multiplex immunoassay. Results: Ec LPS, Scyj LPS, and Scyo LPS stimulated concentration-dependent release of (a) Pro-inflammatory cytokines: IL-6>TNF-; (b) Pro-inflammatory chemokines: MIP-2 (CXCL-2)>MIP-1 (CCL3)>CINC-1 (CXCL-1); and the (c) Anti-inflammatory cytokine: IL-10, at > than 0.1ng/mL Ec LPS and 104ng/mL Scyj and Scyo LPS. Conclusions: After an 18 hour *in vitro* stimulation Scyj and Scyo LPS stimulated classical and alternative activation of rat brain microglia, but both cyanobacterial LPSs were considerably less potent than Ec LPS. Support by the Biomedical Sciences Program, College of Health Science, Midwestern University and the University of Hawaii at Manoa is gratefully acknowledged.

1925 Utility of the Influenza Host Resistance Model for Clinical Risk Assessment

J. Birkebak[2], R. Mikkelsen[1], F. Burleson[1] and L. A. Burns-Naas[2]. [1]*Burleson Research Technologies, Inc, Morrisville, NC and* [2]*Gilead Sciences Inc, Foster city, CA.*

The rat influenza host resistance model is a holistic model that evaluates all components of the immune response. Highly immunosuppressive agents have been shown to have pronounced effects in this model. There is limited published information on more modest immunomodulatory drugs. To evaluate the model's use for clinical risk assessment, 2 marketed immunomodulatory drugs labeled with an increased risk of serious infections were assessed. Female rats were administered tofacitinib (TOF) orally at 0, 1 or 10 mg/kg BID. These doses provided exposures similar to or ~20-fold a clinically efficacious dose. Etanercept (E) was given SC 3x/wk at 10 mg/kg. Dexamethasone (DEX; 2→0.6 mg/kg/day PO) served as the positive control. Agents were dosed 1 week prior to intranasal infection and throughout the study. Rats were evaluated for viral titers, T cell-dependent antibody response (TDAR), lymphocyte levels, cytokine production, macrophage phagocytosis and respiratory burst, and natural killer (NK) cell and cytotoxic T cell (CTL) activity. TOF was associated with a dose-dependent decrease and delay in influenza viral clearance as well as a dose dependent reduction in NK function, and TDAR. At 10 mg/kg BID TOF, there was reduced NK cells and CTL function. Viral clearance was decreased but not delayed with E, and minimal reduction in Day 21 influenza-specific IgG production occurred. DEX produced the expected immunosuppressive response. While the influenza host resistance assay was able to detect an effect of TOF and E on viral clearance, the effect was modest even with a supratherapeutic dose. The model is helpful in confirming a potential clinical risk exists (hazard identification) for compounds shown to be immunomodulatory in more traditional toxicology studies. This model, as with many preclinical models, may not be able to discriminate between small incremental increases or decreases in clinical risk. The utility of the model for rank-ordering drug candidates in early development or for the evaluation of combination therapies needs further evaluation.

1926 TDAR: A Study Comparing Different Antigen (KLH) Supplier in Rats

C. Dumont, M.-H. Côté, C. Parente and M.-S. Piché. *Immunology, Charles River, Senneville, QC, Canada.* Sponsor: M.-S. Piché.

T-dependent antibody response (TDAR) to a known antigen (Keyhole limpet hemocyanin) is routinely used in safety testing for the preclinical phase of drug development. KLH is a standard antigen known to mount a robust antibody response, which can provide insightful information on the immunological competence of an animal following administration of a drug. The purpose of the study was to determine if different suppliers, providing KLH products with lower endotoxin and bioburden levels, could be used to replace our standard product and if the responses mounted from these "cleaner" KLH products are equivalent. We compared the TDAR using KLH from 4 different suppliers in Wistar-Hannover rats at two different doses (300 and 1000 µg/animal, i.v.). The KLH from one of these suppliers was used as a standard product in several different laboratories, including ours. The KLH previously used was in a whole protein format, while the product tested from the 3 new suppliers included two whole protein products and one KLH subunit product. Detectable anti-KLH antibody responses were measured with all products in whole protein format, while weak responses were detected with the KLH subunit product. The data demonstrated that there were similarities in the magnitude of the anti-KLH IgM and IgG responses mounted following immunization with different KLH products (whole protein only). This study therefore suggests that different KLH suppliers may be used for TDAR studies, some of which having the added benefit of administrating a product with lower endotoxin and bioburden levels to animals.

1927 Detection of IgG and IgM Responses to Hepatitis B Antigen in Cynomolgous Monkey

G. Ferency[1], G. Rao[2], O. Amy[2], J. Olsen[1], J. Puchalski[1] and A. M. Frantz[1]. [1]*Covance, Inc., Madison, WI and* [2]*Genentech Inc., South San Francisco, CA.*

The T-cell dependent antibody response (TDAR) assay has become a standard approach to assess immune function. The successful TDAR is dependent on multiple functional immune processes. Antigen uptake and presentation is followed by T cell recognition and B cell activation to produce antibodies. Alteration of any of these processes will result in abnormal antibody production and indicate the occurrence of immunomodulation. For this reason, the TDAR assay provides a good marker of adaptive immune system function. Sheep red blood cells (sRBCs), tetanus toxoid (TT), and keyhole limpet hemocyanin (KLH) are well-established as T-dependent antigens used in pre-clinical safety assessment. However, these assays are not useful for assessment of human immunomodulation. Because of this, the ability to predict the effect of a drug on the human immune system is further complicated. Hepatitis B surface antigen (HBsAg) is a clinically used human vaccine and also a T-dependent antigen that, unlike sRBCs, TT, and KLH, can be used in both pre-clinical and clinical safety assessment. The ability to translate an assay from animal models to a human model may enhance the predictability of immunomodulation of drugs at the clinical level. In a pilot study for an ELISA-based assay for measurement of HBsAg IgG- and IgM-specific antibodies, cynomolgus monkeys were immunized with HBsAg (Engerix B [HBsAg] at the 20 µg adult human dose) and re-challenged with a second vaccination 28 days later. Blood samples were collected prior to vaccination and weekly for eight weeks to assess the kinetics of the antibody response. Serum samples were diluted 1:100 and titrated 5x to a final dilution of 1:8100 prior to being incubated with plate-bound HBsAg (HBsAg Ig Detection Kit; Alpha Diagnostic Intl Inc.) Bound antibodies were detected with horseradish peroxidase-conjugated goat anti-human IgG or IgM. After substrate addition, colorimetric analysis was used to calculate an interpolated cutpoint titer. After the initial immunization, anti-HBsAg IgG- and IgM-specific primary antibody peak responses occurred at Day 22 and Day 15 respectively. After the re-challenge, anti-HBsAg IgG secondary response continued to increase. Planned future experiments include addition of an immunosuppressant to verify TDAR effects and vaccine dose optimization to assess the ability of the HBsAg TDAR assay to detect immune enhancement as well as immunosuppression.

1928 Investigation of KLH (Keyhole Limpet Hemocyanin) Antigen Dose Response in Male and Female Cynomolgus Monkeys to Establish an Optimal Response to Study Immune Enhancement

C. M. Satterwhite[2], R. W. Comba[2], A. Oldendorp[3] and R. Prell[3]. *[1]gRED Nonclinical Operations, Genentech, Inc., SO. San Francisco, CA; [2]Laboratory Sciences, Charles River Laboratories, Reno, NV and [3]Safety Assessment, Genentech, Inc., SO. San Francisco, CA.*

An increasing number of biotherapeutics are in development for which the mechanism of action is the stimulation of antigen specific T-lymphocyte responses against cancer antigens. The T-cell dependent antibody response (TDAR) has historically been used to evaluate the immunotoxic potential of compounds as a result of immune suppression. The aim of this study was to develop and characterize an antigen challenge model in cynomolgus monkeys which is sensitive to immune enhancement and enable the use of the model to investigate PK/PD/safety relationships for future immunostimulatory (i.e. cancer immunotherapy) compounds. TDAR responses were evaluated in cynomolgus monkeys (2/sex/group) after immunizations with KLH at 0.1 mg, 1.0 mg, and 10 mg/animal via intramuscular injection (IM) on Days 1 and 36. Samples were collected for the assessment of humoral and cell mediated immune responses via serum anti-KLH IgM and IgG titers and cytokine release (plasma cytokines, intracellular cytokines, and *ex vivo* ELISPOT) respectively. A dose response to KLH immunization was detected via the anti-KLH IgM and IgG antibody responses. Similarly, increases in IFN-γ, IL-2 and IL-4 producing cells were detected via ELISPOT analysis, on Day 7 and peaked on Day 35 following the primary KLH challenge. There were no alterations in the frequency or number of T-lymphocyte populations after immunization with KLH, or the production of intracellular IL-2, IL-4, IFN-γ or TNF-α by T-helper lymphocytes after *ex vivo* KLH restimulation. These data provide the foundation for future studies which will assess the impact of KLH dosing in the presence of immunostimulatory compounds, and demonstrate the utility of incorporating T-cell specific immunoassays to better assess antigen-specific responses after immune stimulation.

1929 Development of a Flow Cytometric Method to Measure Neutrophil Precursors in Cynomolgus Monkey

R. Magpantay and J. R. Piccotti. *Exploratory Toxicology, Celgene, San Diego, CA.*

Neutropenia is often an unwanted side effect of immunomodulatory and anticancer agents. One possible mechanism of toxicity is a direct effect on the maturation of neutrophil precursors in bone marrow. Although characterization of neutrophil lineage in human bone marrow by flow cytometry is well-documented, limited information exists on the appropriate surface markers to detect neutrophil precursors in monkey bone marrow. The current study was conducted to develop a flow cytometric assay to delineate neutrophil subpopulations in bone marrow of cynomolgus monkey. Bone marrow cells obtained from the femur were stained with fluorochrome-conjugated antibodies specific for CD33 and CD11b. CD33 is reported to be highly expressed on human neutrophil blasts, promyelocytes and myelocytes, and to a lesser extent on metamyelocytes, bands and mature neutrophils. CD11b is absent on human blasts and promyelocytes, but expressed on myelocytes through mature neutrophils. Since the expression of CD33 and CD11b on cynomolgus monkey neutrophils at various stages of development has not been reported, neutrophil subpopulations in this study were bucketed into three categories: "immature" (CD33+/CD11b-), "intermediate" (CD33+/CD11b+) and "mature" (CD33-/CD11b+). We found the relative percentages of CD33+/CD11b-, CD33+/CD11b+ and CD33-/CD11b+ neutrophils to be 4-6%, 15-30% and 1-11%, respectively, of the total bone marrow cell population. A distinct CD33+/CD11b- population was observed. However, a continuum of staining was seen on CD33+/CD11b+ and CD33-/CD11b+ cells, consistent with the reported expression pattern of these markers on human neutrophils. The results of this study demonstrated that measuring CD33 and CD11b cells on monkey bone marrow cells can be used to characterize neutrophil precursors. A notable assumption made in this study was the expression of CD33 and CD11b on cynomolgus monkey neutrophils is consistent with human neutrophils. Further investigations are ongoing to compared CD33/CD11b expression by flow cytometry with cell morphology in order to test this conclusion.

1930 A Novel Approach of Assessing Preclinical Immunotoxicity and Lead Optimization of Antibody-Drug Conjugates Using Flow Cytometry in Cynomolgus Monkeys

R. Tadagavadi[2], J. England[2], L. Manson[2], J. Hyde[2], J. Setser[2], J. Mertens[2], E. v. d. Horst[1] and N. Makori[2]. *[1]Igenica Biotherapeutics, Burlingame, CA and [2]WIL Research Laboratories, Ashland, OH.*

Antibody-drug conjugates (ADCs) are a new class of biopharmaceuticals designed as a targeted therapy for the treatment of cancer. ADCs are complex molecules composed of monoclonal antibodies conjugated to cytotoxic drugs through chemical linkers with labile bonds. Internalization of ADC bound to specific cell leads to release of the cytotoxic drug and death of target cells. Because of their complex structure, the preclinical development particularly the immunotoxicity assessment of ADCs requires a specialized approach that addresses all of its components: the biologic antibody, the small molecule drug and the linker. In this study, we developed an *in vitro* assay that determines ADC binding to different subsets of blood leukocytes, and then correlated those findings to ADC *in vivo* effects on leukocytes in cynomolgus monkeys. In this lyse/no-wash flow cytometry performed in 96 well plates, fluorescent conjugated ADC was incubated with blood leukocytes and examined for ADC associated median fluorescence intensity (MFI) over its corresponding isotype ADC. Virtually all subsets of leukocytes tested showed binding to ADC with higher MFI as compared to isotype control ADC. To exclude the possibility of ADC Fc fragment, drug conjugate and/or linker binding to leukocytes instead of ADC antigen binding Fab fragment, we conducted a neutralization assay using specific peptide that was used to generate ADC. Incubation of ADC with the specific peptide prior to staining blood leukocytes inhibited ADC binding to T cells, B cells and monocytes. These studies suggest that the binding of ADC to leukocyte subsets is specific and is mediated through Fab fragment of ADC. In addition, the effects of this ADC were assessed in cynomolgus monkeys by immunophenotyping blood leukocytes at pre-dose, day 2, day 7 and day 21 after a single intravenous dose of ADC administration. As expected, ADC treatment caused dose dependent depletion of B cells, T helper and T cytotoxic cells with maximum depletion observed on day 7. Leukocytes gradually replenished to pre-dose levels by day 21. The initial *in vitro* screening of ADC binding to leukocytes employed in this study provides a novel high throughput platform for screening ADCs in lead optimization that can be readily and cost-effectively be applied in drug discovery.

1931 Experimental Procedures Increase the IL6 and Stress Markers in Cynomolgus Monkey

A. Sakai, M. Izumi, T. Minami, T. Komaki, N. Matsumoto, T. Asahi, K. Yunomae and R. Nagata. *Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories (SNBL), Ltd., Kagoshima, Japan.*

Object: Serum cytokines are generally evaluated as immunotoxic biomarkers in toxicity studies for biomedicines. Although stress increases IL-6, there are only a few reports in which effects of experimental procedures on IL-6 have been evaluated. In the present study, effects of experimental procedures (retention, intravenous dosing, and frequent blood sampling) on serum IL-6 and plasma stress markers were evaluated in cynomolgus monkeys. Materials and Methods: Three kinds of procedures were used on a total of 17 male cynomolgus monkeys: [1] retention for 60 minutes (Group 1, 5 males), [2] intravenous infusion of physiological saline (2.5 mL/kg) with 60-minute retention (Group 2, 6 males), [3] frequent blood sampling (6 points) in addition to Group 2 conditions (Group 3, 6 males). Blood sampling points for cytokine analysis were: 0, 4, 8 hours after corresponding retention/dosing time in the acclimation period, and 0, 4, 8, 24, 48, and 72 hours after retention/dosing time in the retention/dosing period. Parameters were cytokines (IL-2, IL-4, IL-5, IL-6, TNF, and IFN-γ, BD CBA kit), stress markers (cortisol and cortisone, LC-MS/MS), and blood chemistry (22 parameters) Results: In comparison with the values in the acclimation period, IL-6 and stress markers increased immediately after retention, and LDH and CK were increased 4 and 8 hours after retention in Group 1. These increases were considered to be due to the retention procedure. No differences were noted in IL-6, stress markers, LDH, or CK between Groups 1 and 2. IL-6, LDH, and CK in Group 3 increased but stress markers did not when compared with Group 1 or 2. All other cytokines except IL-6 were below the lower limit of quantification. Discussion: The 60-minute retention itself increased the serum or plasma levels of IL-6 and stress markers. Frequent blood sampling following retention/dosing further increased IL-6. In conclusion, the effects of retention and blood sampling should be considered in monkey studies which evaluate IL-6 and stress markers.

1932 Cigarette Smoke Mediated Regulation of Autophagy in Raw 264.7 Macrophages

P. Bagam and S. Batra. *Environmental Toxicology, Southern University A&M College, Baton Rouge, LA.* Sponsor: S. Batra.

Chronic obstructive pulmonary disease (COPD) is a major global public health problem. Although the mechanisms responsible for the pathogenesis of COPD remain unclear, there is no doubt that exposure to cigarette smoke (CS) is a major risk factor. CS induces oxidative stress in lungs, resulting in cell death and senescence. Autophagy, once considered as a basic catabolic process to remove damaged organelles and proteins, has now evolved into a highly regulated process capable of specificity. There is however, paucity of information regarding the role of autophagy in COPD. Autophagic process is mainly executed by a series of autophagy related proteins (ATGs) regulated by Fork head box class O (FOXO) transcription factor. LC3 is an autophagy marker which conjugates to phosphatidylethanomine (PE) that requires ATG7. The lipidated form of LC3 II is attached on to the autophagosome membrane. To determine the effect of CS on induction of autophagy we challenged RAW 264.7 murine macrophages and THP1 cells with CS condensate or DMSO (control) for 24h. We determined the expression of LC3 & ATG7 by immunofluorescence and western blotting. We observed increase in the expression of ATG7 and cleavage of LC3-I to LC3-II in CS challenged cells. The expression of ATG7 is important for recruitment of LC3 (ATG8) to the autophagic complex. Our results warrant for further investigation of the upstream regulators particularly at post- translational levels in response to CS exposure. These findings suggest that autophagy may play critical role in disease pathogenesis of COPD.

1933 Establishment of Cell Based Model for Studying Human Angiotensinogen Gene Regulation After Xenobiotics

C. M. Menezes[2] and R. A. Ansari[1]. [1]*Pharmaceutical Sciences, Nova Southeastern University, Fort Lauderdale, FL and* [2]*Pharmaceutical Sciences, Universidade Federal da Bahia, Salvador, Brazil.*

Angiotensinogen is a liver secreted protein which plays a significant role in blood pressure regulation and mediate fibrogenic transformation of liver after hepatotoxicants mediated liver injury. The regulation of human angiotensinogen differs from mouse and rats. Using the human angiotensinogen transgenic animals for studying its regulation is costly. Alternatively, we have demonstrated earlier that human hepatocytes can be used for studying regulation of human angiotensinogen after treatment with ethanol. Since hepatotoxicants exposure induce immune response due to liver injury, we have earlier studied the effects of interleukins (IL-1β, and IL-6) and phorbol (PMA) esters on the secretions of AGT from HepG2 and Huh7 cells. Treatment of these hepatocytes resulted into increased secretion in media after treatment with IL-1β, IL-6 and PMA after 6 and 12 hrs. The effects of these treatments on the cellular level of AGT were also determined to correlate the secretions with cellular AGT level. It was observed that cells contained 3 forms of AGT. The level of matured AGT in cells was not increased but rather a decreased level was observed in treated hepatocytes. There were two lesser size, unmatured1 and further less size, unmatured 2 AGT. The unmatured form of AGT (unmatured1 and unmatured 2) were slightly increased in treated groups in both cells compared to control. It is concluded that these cells can be utilized for studying the effects of inducers of angiotensinogen.

1934 Nrf2 Promotes Th2 Differentiation in Murine and Human CD4 T Cells

A. E. Turley[3,5], J. W. Zagorski[3,5], C. D. Klaassen[4], P. E. Fields[4] and C. E. Rockwell[3,5]. [1]*Cell and Molecular Biology Program, Michigan State University, East Lansing, MI;* [2]*Dept of Pathology & Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS;* [3]*Dept of Pharmacology and Toxicology, Michigan State University, East Lansing, MI;* [4]*Dept of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS and* [5]*Institute for Integrative Toxicology, Michigan State University, East Lansing, MI.*

Nuclear factor erythroid-derived 2 like-2 (Nrf2) is a transcription factor that regulates a battery of antioxidant and detoxification genes in response to cell stress. Nrf2 is activated by a variety of stressors, including electrophilic xenobiotics such as tBHQ (tert-butylhydroquinone), a commonly used food preservative. CD4 (helper) T cell differentiation is a critical step in the adaptive immune response in which CD4 T cells tailor the response to a specific pathogen. Differentiated CD4 T cells are classified into functional subsets (Th1, Th2, etc.) based on the particular cytokines they express. Although numerous studies have demonstrated that Nrf2 is immunomodulatory in a variety of inflammatory models, the role of Nrf2 in CD4 T cell differentiation remains unclear. Thus, the purpose of the current studies was to determine the role of Nrf2 in primary murine and human CD4 T cell differentiation. Murine or human CD4 T cells were isolated from spleens or blood, respectively, and pre-treated with tBHQ (0.001-1μM) or vehicle (0.01% ethanol) for 30m prior to activation with antibodies against the T cell receptor and a costimulatory receptor. tBHQ treatment increased production of the Th2 cytokines IL-4, IL-5, and IL-13 in human and wild-type, but not Nrf2-null, mouse CD4 T cells. tBHQ inhibited production of the Th1 cytokine IFNγ in wild-type, but not Nrf2-null, mouse CD4 T cells, and had no effect on human CD4 T cells. tBHQ increased GATA-3, and decreased T-bet, DNA binding in wild-type, but not Nrf2-null, mice. Collectively, the results strongly suggest Nrf2 promotes Th2 differentiation in murine and human CD4 T cells. (Supported by NIH grants ES018885 and ES007255).

1935 *In Vitro* Screening Method for Characterization of Macrophage Activation Responses to Environmental Stress

B. W. Lewis, R. Sultana and Y. Saini. *Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, LA.*

Macrophage activation refers to the enhanced functionality of macrophages upon encountering endogenous or exogenous stimuli. Due to the existence of limitless stimuli and a multitude of surface receptors on macrophages, the nature of activation (or acquired functioning) can be specific to the encountering stimulus. In current study, we developed a macrophage-activation screening assay in 96-well format. The assay involves the generation of bone marrow derived macrophages, their activation into two extreme activation states, and screening of activated macrophages for expression of bonafide protein biomarkers. In our screening, M1- and M2-specific markers were included. INOS is the signature M1-activation marker that is characteristic of classical activation response. For M2-specific signatures, we included FIZZ1 and CHI3L/4 markers. FIZZ1, a consistently observed M2 marker, was only seen in M2 macrophages. Although baseline expression of CHI3L3/4 (YM1/2) was observed in M0 (Naïve) as well as M1 macrophages, only M2 macrophages showed upregulated expression. Interestingly, we observed a macrophage activation subtype that shows MMP12 upregulation with no sign of upregulation of INOS and FIZZ1. Therefore, we included four additional activation-associated markers i.e. MMP12, ALOX12/15, ARG1, HIF2, and COX1. Further, after determining antibody specificity, the assay can be modified or expanded based on researchers needs. Currently, we are screening the effect of exposure of environmental toxicants including cigarette smoke extract, nanoparticles, and selective metals on macrophage activation. The goal of this screening includes the analyses of dose and macrophage activation responses and identification of selective biomarkers of toxicity. In conclusion, an assay developed hereby is highly applicable, high-throughput, flexible, and informative. This assay could be adapted for biomarker determination in pathological conditions and toxicant/drug safety screening.

1936 Stress-Mediated Immunomodulation: Metallothionein Decreases SDF-1α Mediated Chemotaxis

S. Bhandari and M. A. Lynes. *Molecular and Cell Biology, University of Connecticut, Storrs, CT.*

Metallothionein (MT) is a small molecular weight stress response protein that is highly conserved across a wide range of species. The expression of MT increases under stressful conditions such as exposure to heavy metals, reactive oxygen and nitrogen species, inflammation, and infection. MT has high affinity for divalent heavy metals such as zinc, copper, mercury and cadmium and thus is central to the maintenance of intracellular metal homeostasis and as a means to reduce metal toxicity. MT also serves as a regulator of cellular redox potential and as a buffer of free radicals. MT has traditionally been considered to be an intracellular protein, but its presence in extracellular compartments such as serum, urine, milk, prostatic fluids, bile, liver sinusoids, bronchoalveoli, and pancreatic acini indicate a role for this extracellular pool. We have previously shown that MT can bind to the surface of lymphocytes *in vitro*, and is found on circulating lymphocytes in animals that display chronic inflammatory disease. MT (at 7.2 μM-14.3 μM) is a potent chemoattractant for Jurkat T cells (ATCC TIB-152). This effect can be blocked by antagonists of G-protein coupled receptors. When Jurkat T cells are pre-incubated with 14.3 μM MT, there is a significant decline in their chemotactic response towards SDF 1-α. This preincubation does not interfere with internalization of CXCR4, (the SDF-1α receptor), that occurs during SDF-1α-induced chemotaxis. MT mediated chemotaxis is also observed with the human breast cancer cell line, MDA-MB 231 (ATCC HTB-26). The mechanism of

this phenomenon is under investigation. These functional activities of MT suggest an immunomodulatory role for the stress protein in inflammatory disease, and also in the progression of metastatic cell movement. Hence, manipulation of MT represents a possible opportunity for therapeutic intervention in these diseases, and in the management of environmental toxicant-mediated immunomodulation.

1937 Species Differences in the Effects of TCDD on a Transcriptional Regulatory Region within the Ig Heavy Chain Gene

Z. J. Alfaheeda. *PHARMAXOT, Wright State University, Dayton, OH.*

The immunoglobulin heavy chain gene (Igh) is regulated by numerous regulatory elements including the 3'Igh regulatory region (3'IghRR). Several transcription factors are involved in modulating the 3'IghRR including NF-κB, AP-1, and the aryl hydrocarbon receptor (AhR). The AhR is a ligand-activated transcription and it mediates the metabolism of environmental toxicants such as TCDD. TCDD an environmental contaminant and ligand for the AhR, is known to potently inhibit Immunoglobulin (Ig) expression in animal models. In mice, TCDD inhibits 3'IghRR activation in an AhR-dependent manner, which may be directly mediated by binding of the AhR to dioxin response elements (DRE) within the 3'IghRR. Structural differences have been found between the human and mouse Igh genes, including a duplication of the 3'IGHRR in humans as well as a polymorphic region in the hs1.2 enhancer. The polymorphic human hs1.2 enhancer has been correlated with several autoimmune diseases and has been identified as a sensitive target for TCDD in a mouse cell line (CH12.LX) model. Due to species differences in the 3'IghRR structure and TCDD-induced effects on hs1.2 activity, the objective of this study was to determine the transcriptional influence of each human 3'IGHRR enhancer and sensitivity of these enhancers to TCDD. Using luciferase reporters regulated by the individual 3'IGHRR enhancers or in combination and cellular models that include the well characterized mouse CH12.LX B cell line and the CL-01 human B cell line, which can be induced to secrete Ig and CSR, the effect of cellular stimulation and TCDD on enhancer activity was studied. Prominent species differences were found including a lack of activation by cellular stimulation and a less consistent effect of TCDD on the human 3'IGHRR as compared to the mouse 3'IghRR. A better mechanistic understanding of the 3'IGHRR is important to elucidating its role in autoimmune disease and potentially the development of novel therapeutics.

1938 Reducing the Number of hs1.2 Invariant Sequence Repeats in the 3' Immunoglobulin Heavy Chain Regulatory Region Alters Ig Expression

A. Snyder, N. Panstingel, S. Abdullah and C. Sulentic. *Department of Pharmacology and Toxicology, Wright State University, Dayton, OH.*

The immunoglobulin heavy chain (IGH) locus is partially responsible for immunoglobulin (Ig) production in B cells. The human IGH locus contains two 3' regulatory regions (3'IGHRR) that each contain three enhancers, which help drive overall transcription of the locus and also influence class switching to different Ig isotypes. The hs1.2 enhancer within the 3'IGHRR is polymorphic in humans, containing a 53 bp invariant sequence (IS) that can be repeated up to four times. The hs1.2 enhancer is a sensitive target of exogenous chemicals, particularly 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent inhibitor of Ig expression in animal models. The IS polymorphism has also been associated with many immunological disorders in human patients. Therefore, understanding the immunological role of the hs1.2 polymorphism could be invaluable to human health. Previous studies of the human 3'IGHRR enhancers have used luciferase reporter plasmids to evaluate their activity, but those studies may not accurately reflect the region's function due to removing the regulatory elements from their native context. In this study, the CRISPR/Cas9 genetic editing system was used to modify the hs1,2 enhancer in the human 3'IGHRR. Using a single CRISPR/Cas9 plasmid targeting the polymorphic hs1,2 invariant sequence (IS) repeats in a human B cell line has successfully resulted in clonal populations containing a reduced number of IS repeats. In fact, cellular populations exhibiting virtually every possible combination of polymorphic hs1.2 alleles have now been created using this system. Recent experiments with these cells have shown that reducing the number of IS repeats also reduces expression of some Ig isotypes. The response to B-cell stimulation is also greatly reduced in cells with fewer IS repeats. Additionally, cells with fewer IS repeats appear less sensitive to TCDD-induced inhibition of Ig expression. As more hs1,2 modified cells are analyzed, valuable information regarding the function of the hs1,2 alleles in human health and susceptibility to exogenous chemicals will be revealed.

1939 Immunogenicity of Green Fluorescent Protein and the Disruption of the Immunodominant T Cell Epitope

T. Eyes, A. Doig, I. Kimber and R. J. Dearman. *Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.*

The discovery and application of Green Fluorescent Protein (GFP) has made a very significant contribution to biological research. Labelling cells with a genetically encoded fluorescent signal has proven to be an extremely powerful tool for monitoring biological events both *in vitro* and *in vivo*. However, there is strong evidence that enhanced GFP (EGFP, commonly used variant) can elicit an immune response when used in animal models which can have serious consequences for the integrity and reliability of experimental studies, including the rejection and elimination of cells expressing EGFP. The aim of these experiments was to develop an EGFP construct with reduced immunogenicity in the BALB/c model strain but which maintains fluorescent functionality. The major histocompatibility complex (MHC) class I immunodominant epitope of EGFP in BALB/c mice has been described previously. EGFP was expressed in Escherichia coli and saturation mutagenesis was used to generate a library of EGFP variants where the MHC class I anchor site in the dominant epitope was mutated. The library was screened for fluorescent EGFP variants which possessed anchor site mutations that based on computational prediction should display reduced immunogenicity. The EGFP mutation tyrosine 200 to threonine (Y200T) variant that expressed near identical fluorescent properties to the parent EGFP was selected and murine A20 B cell lymphoma A20 cells were stably transfected with either EGFP wild type (WT) or Y200T. To investigate cell associated immunogenicity, female BALB/c mice were immunised by sub cutaneous injection with 1x106 or 1x107 cells expressing EGFP or EGFP Y200T and serum analysed at day 14. The Y200T mutation significantly reduced the anti-EGFP IgG response; mean titers of 1/512 (WT) compared to 1/83 (Y200T, $P<0.05$). Interestingly, immunisation with recombinant WT or Y200T EGFP protein each resulted in relatively high levels of IgG antibody, with similar titers. These data indicate that through a single mutation the immunodominant T cell epitope in EGFP can be disrupted and tolerance to cell associated protein enhanced in mice. This presents a novel strategy for improving tolerance to heterologously expressed proteins without the requirement for immunosuppression.

1940 Retinoic Acid and IFNg Induce Parallel Arginase-1 and Nos-2 Gene Expression in Primary Mouse Kupffer Cells

I. Mohar[1,2] and I. N. Crispe[2]. [1]*Gradient, Seattle, WA and* [2]*University of Washington, Seattle, WA.*

Macrophage polarization is an important determinant of pathologic and immunologic outcomes, because macrophages help maintain tissue homeostasis and direct differentiation of helper lymphocytes. Acute inflammation is associated with classically activated inflammatory macrophages, while chronic inflammatory diseases are associated with fibrosis and alternatively activated macrophages. Liver fibrosis is a common complication of chronic liver inflammation, yet the molecular signals that promote alternative activation of Kupffer cells are poorly defined. Fibrosis is associated with accumulation of myofibroblasts derived from activated pericytes. In the liver, hepatic stellate cells are the resident pericytes that release retinoic acid following activation. We tested the hypothesis that retinoic acid (RA) polarizes Kupffer cells to an alternatively activated state. We contrast this proposed mechanism to activation by interferon-gamma (IFNg) or lipopolysaccharide (LPS). Kupffer cells were isolated from C57BL/6J mice by FACS-based purification; plated at 30,000 per well for 24 hours; and then stimulated for 24 hours with 100 ng/mL LPS, 100 ng/mL IFNg, 100 μM RA, or IFNg + RA. Isolated RNA was analyzed by microfluidic quantitative real-time-PCR, using assays for genes characteristic of macrophage activation. LPS induced Socs3, Ptgs2, Nos2, Tnf, Icam1, Il6, Il1a, Il10, and Marco. IFNg induced Socs3, Ptgs2, Nos2, Tnf, Icam1, Ciita, Ccl12, and Klf4 and suppressed Mrc1. Retinoic acid induced expression only of Arg1. Unexpectedly, co-treatment with RA and IFNg induced an additive gene expression pattern. In summary, treating primary Kupffer cells with retinoic acid induces Arg-1; co-treating with IFNg and RA simultaneously induces Arg1 and Nos2; and LPS (but not IFNg) induces expression of Il6. These results support the hypothesis that activation of hepatic stellate cells may induce aspects of alternative activation in Kupffer cells, and they demonstrate that classic and alternative activation fates of macrophages are not always mutually exclusive.

1941 Development of a Co-culture System for Inhalative Therapeutics Against *Pseudomonas aeruginosa* Infections

L. Boge[1], S. Häußler[2], J. Knebel[1], M. Müller[1] and S. Wronski[1]. [1]*Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany and* [2]*Twincore, Center for Experimental and Clinical Infection Research, Hannover, Germany.* Sponsor: C. Dasenbrock.

Background: Persistent infection with *Pseudomonas aeruginosa* is a major cause of morbidity in cystic fibrosis patients. One of the main virulence mechanisms of *P. aeruginosa* is the ability to form biofilms, which leads to a high tolerance towards and diminished efficacy of antibiotic treatment. One concept for therapeutic improvement is the application of aerosolized therapeutics. This project aims to develop a co-culture model of human airway epithelial cells and *P. aeruginosa* for exposure to novel aerosolized compounds for inhalative therapy. Methods: Air-liquid-interface cultures of A549 human epithelial cells or human primary cells were infected with *P. aeruginosa* PAO1 or a clinical isolate known to grow small colony variants. Cell vitality was measured 6h and 24h post infection by life/dead staining (Calcein AM and Ethidiumhomodimer1) and analyzed via laser scanning confocal microscopy. The fluorescence signal was quantified via photometry. Cytokine-release (Il-8) was measured after bacterial infection using ELISA and bacterial load determined by dilution plating and counting of colony-forming units. Life/dead (Syto9 and Propidiumiodid) and matrix staining (ConcanavalinA) were used to visualize the vitality and the structure of the bacterial biofilm via confocal laser scanning microscopy. Results: Sustained viability of the cells with an increased cytokine release of infected cells was observed up to 24h. This setup now enables the investigation of bacterial sensitivity against antibiotic treatment under conditions occurring in persistent infection. Conclusion: Short term co-cultivation of *P. aeruginosa* and human epithelial cells was successfully established and can be used for parallel efficacy and toxicity testing of novel anti-infective compounds. Further work will focus on *in vitro* exposure towards inhalative compounds for preclinical efficacy and toxicity testing.

1942 Compensatory Restitution by Nsaid-Induced Protein Against Intestinal Ulcerative Insults

S.-H. Park[2], H. J. Choi[2], K. H. Do[2], J. Kim[2], M. Yu[2] and Y. Moon[1,2]. [1]*Immunoregulatory Therapeutics Group in Brain Busan 21 Project, Yangsan, Korea, Republic of and* [2]*Department of Biomedical Sciences, Pusan National University School of Medicine, Yangsan, Korea, Republic of.*

In response to ulcerative mucosal injuries, intestinal epithelial restitution is a critical event in the early defense against harmful attacks by luminal antigens. Based on the assumption that epithelial NSAID-induced Protein (NiP) is an endogenous regulator of ulcerative stress-induced injuries, the expression and functions of NiP were investigated. Genetic ablation of NiP decreased murine survival from the intestinal ulcer, confirming protective roles of NiP in ulcerative insults. Moreover, enhanced expression of NiP during the wound healing process was associated with epithelial cell migration and spreading. In response to ulcerative injury, RhoA GTPase, a cytoskeleton modulator, mediated epithelial restitution via enhanced motility. RhoA expression was prominently elevated in the restituting epithelia cells around the insulted wound bed and was attenuated by NiP deficiency. Pharmacological intervention with RhoA thus attenuated NiP-mediated epithelial migration during epithelial restitution. Taken together, epithelial restitution was promoted by enhanced NiP expression and subsequent enterocyte locomotion during the wound healing process, suggesting clinical usefulness of NiP as a novel endogenous muco-protective factor or an indicator of therapeutic efficacy against the ulcerative gastrointestinal diseases including IBD (This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health &Welfare, Republic of Korea (HI13C0259)).

1943 Dysregulated Immune System Networks as an Outcome of Altered miRNA Expression and DNA Methylation in War Veterans with Posttraumatic Stress Disorder

M. Bam, X. Yang, P. Nagarkatti and M. Nagarkatti. *Pathology Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC.*

Posttraumatic stress disorder (PTSD) is a psychiatric disorder and manifests a chronic systemic inflammation in the patients, in addition to other symptoms. However, the molecular mechanisms involved in the inflammation remain poorly understood. Previous studies employing transcriptomic data aimed to identify individual genes rather than identifying gene sets for different biological pathways. Moreover, the mechanisms regulating the expression of the dysregulated genes involved in inflammatory pathways in PTSD are not reported adequately. Through transcriptome sequencing and micro-RNA (miRNA) microarray, we identified 326 dysregulated genes and 190 miRNAs in the PBMCs of PTSD patients. By performing expression pairing of the dysregulated genes and miRNAs, we demonstrate an inverse relationship in expression of miRNAs and their target genes. Functional analysis of the dysregulated genes obtained after expression pairing, indicated a strong alteration of the canonical pathways specific to immune system biology in PTSD. DNA methylation analysis of promoter regions of several dysregulated genes also pointed to differences between control and PTSD patients, again indicating a role of this epigenetic mechanism in PTSD inflammation. Overall, combining data from RNA-seq with miRNA microarray and DNA methylation provided a holistic view of several pathways in which the dysregulated genes result from altered epigenetic mechanisms controlling their expression in PTSD. Thus, we conclude that analysis combining transcriptomic data with miRNA array and DNA methylation data can provide key evidence for dysregulated pathways and the regulatory mechanisms at the molecular level in PTSD and other diseases. Most importantly, the present data further provides strong evidence that PTSD manifests an inflammatory condition which is epigenetically regulated (This work was supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, and P20GM103641 to PN and MN).

1944 Inhalation of Chemicals - Subjective Irritative Symptoms versus Inflammatory Markers in Plasma

L. Ernstgård, M. Bottai, G. Johanson and B. Sjögren. *Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

Inflammation is a complex response to physical, chemical and biological stimuli. Inhalation of airborne chemicals may cause irritation and inflammation in the airways resulting in an increase in inflammatory markers. Approximately one third of the occupational exposure limits are based on irritation as the critical effect. We carried out a pooled analysis of the measurements chamber studies where volunteers had inhaled vapors of acetic acid, acrolein, 1,4-dioxane, n-hexanal, hydrogen peroxide, m-xylene, 2-propanol, or mineral spirit (white spirit, standard or dearomatized) at levels equal to or below the 8-h Swedish occupational exposure limit (OEL). Symptom ratings with visual analog scales (eye, nose and throat irritation, smell), and objective measurements (blink frequency by EMG, nasal swelling by acoustic rhinometry, FEV1 by spirometry) were compared with the inflammatory markers high sensitive C reactive protein (HsCRP), serum amyloid A protein (SAA), and interleukin-6 (IL-6). The exposures were generally too low to cause significant irritation or inflammatory response. This is not surprising, as the OELs are set to avoid adverse effects. However, logistic quantile regression analyses revealed a negative association between rated irritation and HsCRP, suggesting a down-regulation of HsCRP after short-term exposure to chemical vapor. This reaction may be an example of the cholinergic anti-inflammatory pathway, mediated by inhibition of the inflammatory response by vagal stimulation.

1945 Exposure to Urban Dust Particles *In Vitro* and *In Vivo* Enhances Th17-Associated Cytokine Expression

J. H. Fechner[3], G. Hautenhauer[3], A. Rodridguez[1], C. A. O'Driscoll[3], X. Zhang[3] and J. D. Mezrich[3]. [1]*Medical College, University of Pueto Rico, San Juan, PR;* [2]*Neuroscience, University of Wisconsin, Madison, WI and* [3]*Surgery, University of Wisconsin, Madison, WI.* Sponsor: C. Bradfield.

Background: Airborne pollution is a known risk factor for autoimmune diseases and diseases of the lung such as asthma. The mechanisms underlying the effects of pollution in these diseases are not fully understood. Our lab recently reported on an aryl hydrocarbon receptor (AHR)-dependent increase in Th17 differentiation *in vitro* following exposure to SRM1649b, a standardized reference urban dust particles (UDP). To further investigate these findings, OT-II mice, transgenic C57BL/6 mice which have CD4 T cells that exclusively express a single TCR specific for an ovalbumin, were utilized. Methods: For *in vitro* experiments, UDP or PBS vehicle was added to either OT-II splenocytes cultured in the presence of ovalbumin or naïve OT-II T cells cultured under Th17 conditions. After 4 days, cells were analyzed for relative mRNA expression of TH17-associated cytokines IL-17A and IL-22 as well as Cyp1A1, a marker of AHR activation. IL-17 expression was measured by ELISA using culture supernatants or by intracellular cytokine staining and flow cytometry. Preliminary experiments were conducted using the neutrophilic airway inflammation model (NAI). OT-II mice (n = 3 per group) were treated intranasally with ovalbumin +/- UDP for 3 consecutive days. Twenty-four hours after the last dose, mice were euthanized and bronchoalveolar lavage fluid (BALF) was collected for measurement of IL-17A and IL-22 protein levels by ELISA. Results: The addition of UDP significantly increased IL-17A, IL-22 and Cyp1A1 mRNA expression compared to control in both splenocytes and TH17 cultures. These results parallel our previously published data using B6 lymphocytes. In TH17 cultures, IL-17A levels were 50% higher in supernatants of cells exposed to UDP compared to PBS-treated controls. Likewise, the fraction of IL-17A expressing OT-II cells was 50% higher in UDP-treated cultures. Finally, in the NAI model, IL-22 expression in BALF was found to be significantly higher in mice co-treated with UDP. BALF IL-17A levels approached significance ($p = 0.061$). Conclusions: These preliminary experiments demonstrated that exposure to UDP can increase Th17 associated cytokines *in vitro* and *in vivo*. Further experiments will be required to determine whether these results are mediated through the AHR of effector T-cells.

1946 Exposure to Benzo(a)pyrene Results in Suppression of FcgRII (CD32) Antibody Binding by Disrupting Lipid Raft Membrane Integrity

C. S. Ryan[3], P. T. Samuel[3], B. Booker[3], A. Ramesh[3], T. Zhang[4], A. Shanker[3], M. Maguire[3] and D. B. Hood[4]. [1]*Department of Biochemistry, Meharry Medical College, Nashville, TN;* [2]*Department of Microbiology, Meharry Medical College, Nashville, TN;* [3]*Department of Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN and* [4]*Division of Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, OH.*

Validation of the basic science research trajectory of our recently described Public Health Exposome framework was accomplished with the identification of a previously uncharacterized molecular mechanism that confers enhanced susceptibility of macrophage membranes to bacterial infection after exposure to the environmental contaminant benzo(a)pyrene, [B(a)P]. The fundamental requirement in activation of macrophage effector functions is the binding of immunoglobulins to Fc receptors. FcgRIIa (CD32a), a member of the Fc family of immunoreceptors with low affinity for immunoglobulin G, has been reported to preferentially bind to IgG within lipid rafts. Previous research suggested that exposure to B(a)P suppressed macrophage effector functions but the molecular mechanism remained elusive. The results from this study describe a mechanism(s) for B(a)P-exposure induced suppression of macrophage function informed in part, by the observed exposure-induced insult on CD32-lipid raft interactions in the regulation of IgG binding to CD32. Moreover, our results demonstrate that exposure of macrophages to B(a)P alters lipid raft integrity by decreasing membrane cholesterol by approximately 25% with a concomitant increase in CD32 into non-lipid raft fractions. This diminution in membrane cholesterol and 30% exclusion of CD32 from lipid rafts effectively causes a significant reduction in CD32-mediated IgG binding to ultimately suppress critical effector functions in the macrophage.

1947 Allergen-Induced T Helper (th) 17 Cell Activation: Comparisons with *In Vitro* Th17 Cells

R. J. Dearman, M. D. Hayes and I. Kimber. *Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.*

Prolonged topical exposure of BALB/c mice to the reference contact allergen 2,4-dinitrochlorobenzene (DNCB), or to the reference respiratory allergen trimellitic anhydride induces selective type 1 or type 2 cytokine secretion profiles, respectively. Furthermore, exposure to DNCB causes the preferential expression of interleukin (IL)-17 cytokines (IL-17A and F and IL-17A/F heterodimer and IL-22). In the current investigations, the pattern of cytokine expression and the requirement for specialized medium (ISCOVES) has been investigated for lymph node cells (LNC) derived from DNCB-activated mice and comparisons made with Th17 cells generated *in vitro*. BALB/c strain mice were exposed topically to 1% DNCB over 13 days and draining LNC isolated. In parallel experiments, CD4+ cells were isolated by negative selection from the peripheral lymph nodes of naïve BALB/c mice and cultured with anti-CD3 and anti-CD28 antibodies and a cytokine cocktail. *In vitro* and *in vivo* derived cells were cultured in standard RPMI medium or in ISOCOVES medium, with and without the selective aryl hydrocarbon receptor (AhR) antagonist (CH-223191; 3 μM) or the natural AhR agonist 6-formylindolo[3,2-b] carbazole (FICZ; 0.3 μM). Cytokine secretion was examined by ELISA. Th17 cells derived *in vitro* expressed ~10ng/ml of IL-17A and heterodimer, ~30ng/ml of IL-17F and considerably lower levels of IL-22 (1-2ng/ml) when cultured in RPMI medium. Culture in ISCOVES medium resulted in a marked (~20fold) increase in all cytokines. Th17 cells derived *in vivo* expressed substantially lower amounts of cytokine, but all at similar levels (~1-2ng/ml) and there was no impact of ISCOVES medium. For both *in vivo* and *in vitro* derived cells, culture with FICZ resulted in a doubling of cytokine levels, irrespective of the culture medium. However, the antagonist was without effect on *in vivo* derived cells whereas cytokine secretion by *in vitro* derived cells was inhibited, particularly for RPMI medium. These data indicate that there are marked differences between the cytokine patterns of Th17 cells produced *in vivo* and *in vitro*, and their requirements for specialized medium. Furthermore, exposure to the contact allergen DNCB stimulates a polarized Th17 cell population which may play a role in the immunopathology of the disease.

1948 Interleukin 1 Cytokine Alone Is Sufficient to Induce Lymphocyte Proliferation

R. J. Dearman and I. Kimber. *Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.*

The murine local lymph node assay (LLNA) is a well-established method for the identification and characterisation of chemicals that have the potential to cause skin sensitisation and allergic contact dermatitis. Skin sensitizing potential is measured as a function of induced proliferative responses in draining lymph node cells (LNC) assessed by incorporation of radiolabelled thymidine. However, there have been concerns raised that certain classes of chemical, particularly surfactants, may be associated with the generation of false positive responses in the assay. It has also been shown that there was a relationship between activity in the LLNA of such presumed nonsensitisers and the release of interleukin (IL)-1α in an *in vitro* skin model. In the current experiments we have therefore explored the ability of cytokine (IL-1α and IL-1β) alone to induce lymph node activation consistent with positive results in the LLNA. BALB/c strain mice (n=3 per group) were injected intradermally with 50 ng per ear of recombinant homologous cytokine suspended in 0.1% carrier protein (bovine serum albumin; BSA) or with BSA alone. Five days later, lymph node activation was assessed as a function of increased cellularity and incorporation of thymidine *in vitro*. Exposure to BSA alone caused a modest increase in both lymph node total cellularity (2-fold) and proliferation (3-fold) compared with naïve controls; however both cytokines stimulated more vigorous activation with 3 to 4-fold increased in cellularity and 8-fold increases in proliferation. Given the small stimulatory effect of BSA alone, in order to control for the injection of this foreign protein, cytokines were suspended in homologous protein (mouse IgG). Mouse IgG alone failed to cause activation whereas parallel treatment with IL-1α resulted in increases in cellularity and thymidine incorporation compared with the controls. These data demonstrate that administration of proinflammatory cytokine alone, in the absence of foreign immunogenic carrier protein, is sufficient to cause lymph node activation and may represent a mechanism whereby some chemicals provoke a positive in the LLNA.

1949 Sparstolinin B, a TLR4-Antagonist Attenuates Early Steatohepatitic Injury in Environmental Toxin Potentiation of Nonalcoholic Steatohepatitis

D. Dattaroy[5], R. K. Seth[5], S. Das[5], F. Alhasson[5], V. Chandrashekaran[5], G. A. Michelotti[1], D. Fan[5], M. Nagarkatti[5], P. Nagarkatti[5], A. M. Diehl[3] and S. Chatterjee[5]. *[1]Metabolon, Durham, NC; [2]Department of Cell Biology and Anatomy, University of South Carolina School of Medicine, University of South Carolina, Columbia, SC; [3]Department of Gastroenterology, Duke University, Durham, NC; [4]Environmental Health Sciences, University of South Carolina, Columbia, SC and [5]Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, University of South Carolina, Columbia, SC.*

Nonalcoholic Steatoheaptitis (NASH), a hepatic manifestation of metabolic syndrome, arises from obesity. Previous studies from others and our laboratory have shown that environmental toxin potentiates NASH progression in obesity. Sparstolonin B (SsnB) is derived from Chinese herb *Sparganium stoloniferum* and is a promising Toll like receptor 4 (TLR4) antagonist. In absence of a proven drug to treat NASH, we hypothesize that SsnB attenuates early steatohepatitic inflammation and injury by inhibiting colocalization of TLR4-Flotillin, decreased Micro RNA 21 and PTEN activation. To prove our hypothesis, we used an early steatohepatitic injury model, where Bromodichloromethane (BDCM), a water disinfection byproduct, was administered for 1 week in diet induced obese mice. A similar group of mice was injected with SsnB for 1 week along with BDCM. Serum ALT- a marker of liver injury was decreased significantly in SsnB treated mice compared to only BDCM-treated mice. Quantitative real time PCR results show that SsnB treatment decreased the gene expression for MCP1, CD68, IL23, IL1β, TNFα compared to only BDCM treated group. We also observed a significant reduction in Micro RNA 21 expression in SsnB treated mice liver compared to mice treated with BDCM only. SsnB treated mice liver showed significantly increased PTEN protein expression compared to BDCM treated mice. Mechanistically, SsnB treatment decreased TLR4 trafficking in lipid rafts as described in tissues and immortalized Kupffer cells. Our previous studies show that NADPH oxidase drives TLR4 trafficking in NASH, we studied the role of SsnB in modulating this pathway. SsnB decreased NADPH oxidase activation invivo and invitro as indicated by decreased peroxynitrite formation. In brief, we report a novel function of SsnB to ameliorate inflammation in early steatohepatitic injury.

1950 FGF21 Mediates Alcohol-Induced Adipose Tissue Lipolysis by Activation of Systemic Release of Catecholamine in Mice

C. Zhao, L. Liu, C. McClain and W. Feng. *Department of Medicine, Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Alcohol consumption leads to adipose tissue lipoatrophy and mobilization of FFAs, which contributes to hepatic fat accumulation in alcoholic liver disease. This study aimed to investigate the role of fibroblast growth factor (FGF)21, a metabolic regulator, in the regulation of chronic-binge alcohol-induced adipose tissue lipolysis. FGF21 KO mice were subjected to chronic-binge alcohol exposure, and epididymal white adipose tissue lipolysis and liver steatosis were investigated. Alcohol exposure caused adipose intracellular cAMP elevation and activation of lipolytic enzymes, leading to FFA mobilization in both WT and FGF21 KO mice. However, alcohol-induced systemic elevation of catecholamine, which is known to be a major player in adipose lipolysis by binding to the β-adrenergic receptor, was markedly inhibited in KO mice. Supplementation with recombinant human FGF21 to alcohol-exposed FGF21 KO mice resulted in an increase in fat loss in parallel with an increase of circulating norepinephrine concentration. Furthermore, alcohol consumption-induced fatty liver was blunted in the KO mice, indicating an inhibition of fatty acid reverse transport from adipose to the liver in the KO mice. Taken together, our studies demonstrate that FGF21 KO mice are protected from alcohol-induced adipose tissue excess-lipolysis through a mechanism involving systemic catecholamine release.

1951 Caspase Inhibition Reduces Pathologic Tissue Factor Driven Procoagulant Activity *In Vitro* and *In Vivo*

A. K. Kopec[4], N. Joshi[4], H. Cline-Fedewa[4], A. P. Spada[1], P. Contreras[1] and J. P. Luyendyk[4]. *[1]Contaus Pharmaceuticals Inc, San Diego, CA; [2]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI; [3]Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI and [4]Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

Tissue factor (TF) is the primary activator of the blood coagulation cascade. Liver parenchymal cells (hepatocytes; HPCs) express TF in a molecular state that lacks procoagulant activity. HPC apoptosis is an important feature of acute and chronic liver diseases. Fas-induced apoptosis increases HPC TF procoagulant activity *in vitro* and HPC TF triggers activation of the coagulation cascade in mice. We determined the impact of a pan-caspase inhibitor, IDN-7314, on TF-mediated coagulation in established experimental settings of Fas-induced hepatocellular apoptosis. Treatment of primary mouse HPCs with the Fas death receptor ligand (Jo2, 0.5 μg/ml) for 8 hours increased HPC TF procoagulant activity and caused a robust release of TF-positive microparticles into the culture medium. Pretreatment with 100 nM IDN-7314 abolished caspase 3/7 activity and significantly reduced both HPC TF procoagulant activity and microparticle release. Treatment of wild-type C57BL/6 mice with a sublethal dose of Jo2 (0.35 mg/kg) for 4.5 hours triggered activation of coagulation, marked by a significant increase in plasma levels of thrombin-antithrombin and TF-positive microparticles. Fibrin(ogen) deposition increased in livers of Jo2-treated mice and localized primarily to the borders of apoptotic hepatocytes, marked by co-staining for cleaved caspase-3. Pretreatment of mice with IDN-7314 largely prevented these procoagulant changes in Jo2-treated mice, in association with a dramatic reduction in caspase-3 activation, hemorrhage/congestion, and hepatocellular injury. Overall, the results indicate a central role for caspase activity in pathologic TF-mediated coagulation following apoptotic liver injury. Moreover, the results suggest the possibility that liver-selective caspase inhibition is a putative strategy to limit procoagulant and prothrombotic changes in patients with chronic liver disease.

1952 Downregulation of Hepatocyte Nuclear Factor 4-alpha as a Mechanism of PFOA- and PFOS-Induced Hepatic Effects

K. M. Beggs, J. N. Lampe and U. Apte. *Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are environmental contaminants that cause hepatomegaly and carcinogenesis in rodents. We investigated the effects of PFOA and PFOS on hepatocyte nuclear factor 4-alpha (HNF4α), using human hepatocytes and occupationally relevant concentrations. PFOA and PFOS both caused a decrease in HNF4α protein without affecting mRNA. RNA sequencing studies revealed that PFOA and PFOS induced significant gene expression changes associated with hepatic steatosis, proliferation and tumorigenesis consistent with a decrease in HNF4α. We further investigated expression of selected HNF4α target genes involved in differentiation and quiescence. PFOA and PFOS decreased expression of CLDN1, ADH1B and TAT genes, which are indicators of hepatocyte differentiation and adhesion. Furthermore, both compounds caused an increase in the expression of promitogenic CCND1. PFOA and PFOS also caused an increase in the mRNA expression of AKR1B10 that is observed in hepatocellular carcinoma, as well as an increase in the protein expression of Nanog, a transcription factor associated with stem cell development. These results indicate a potential for PFOA and PFOS to cause hepatocellular dedifferentiation via HNF4α down-regulation. Finally, *in silico* docking studies were performed to investigate the potential for PFOA and PFOS to interact with HNF4α directly. The highest scoring pose for PFOA occurred in the ligand-binding domain of the crystal structure in almost an identical pose to the native fatty acid ligand. However, PFOS exhibited an alternative docking pose distal from the ligand-binding site. These results indicate a possibility for PFOA or PFOS to interfere with the binding of structurally similar endogenous ligands to HNF4α. Collectively, these data suggest that HNF4α is a cellular target of PFOA and PFOS, and might perpetrate their hepatic effects via down-regulation of HNF4α.

1953 Role of Fibroblast Growth Factor 15 in the Development of High Fat Diet Induced NASH

J. D. Schumacher[2], B. Kong[2], P. Yang[1], L. Zhan[2], R. Sun[1], J. Aa[1], J. Richardson[2], D. Laskin[2] and G. Guo[2]. [1]*Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, China and* [2]*Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

With the rise in obesity in Western civilizations, the prevalence of non-alcoholic steatohepatitis (NASH) is increasing with an estimated 5-10% of the population affected. The primary features of NASH include steatosis, inflammation, and fibrosis often accompanied with metabolic syndrome. Fibroblast growth factor 15 (Fgf15) emerges to be a critical factor in regulating bile acid homeostasis, energy metabolism, and liver regeneration. In order to investigate the effects of Fgf15 on the development of each the listed features of NASH, Fgf15-/- mice were bred into a 75% A129 and 25% C57BL/6 background. Four-week old Fgf15-/- and wild-type (WT) control mice were fed either a high fat diet (HFD) or control diet for six months. A glucose tolerance test was administered during the fifth month. At the end of feeding, liver, intestine, and blood samples were collected for gene expression analysis, determination of serum and tissue lipid composition, identification of biomarker concentrations, and histology. The results showed that HFD-fed Fgf15-/- mice had metabolic syndrome presenting with increased body weight, decreased insulin sensitivity and increased basal total serum cholesterol levels. Knockout animals had altered expression of lipid metabolic enzymes with basal Mtp down-regulated and Acss2 up-regulated while on a HFD compared to controls. Fgf15 deficiency had no observed effects on steatosis as WT and Fgf15-/- mice were found to have comparable hepatic levels of triglycerides and total cholesterol. However, the Fgf15-/- mice were protected from the development of hepatic fibrosis revealed by histology analysis and expression of the genes involved in fibrosis. No changes were observed in hepatic expression of inflammatory genes such as Tnf-α or Icam. Lastly, Fgf15-/- mice fed the HFD had increased bile acid pools and up-regulated gene expression of ileal Ibabp and hepatic Cyp7a1, Cyp8b1, and Bsep compared to WT mice. In summary, during NASH development, Fgf15 deficiency was found to have no effects on liver steatosis or inflammation, however, led to decreased insulin tolerance, increased basal serum total cholesterol levels, altered expression of lipid metabolic enzymes, disrupted bile homeostasis, and decreased liver fibrosis.

1954 RNA Sequencing Reveals Induction of Mmp12 by Fibrin(ogen) Engagement of Leukocyte αMβ2 Integrin as Critical for Liver Repair after Acetaminophen Overdose in Mice

A. K. Kopec[1], N. Joshi[1], H. Cline-Fedewa[1], R. Nault[1], T. Zacharewski[1], M. J. Flick[2] and J. P. Luyendyk[1]. [1]*Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI and* [2]*Division of Experimental Hematology & Cancer Biology, Cincinnati Children's Hospital, Cincinnati, OH;* [3]*Institute for Integrative Toxicology, Michigan State University, East Lansing, MI;* [4]*Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI;* [5]*Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

Liver injury caused by acetaminophen (APAP) overdose is associated with activation of the blood coagulation cascade and deposition of fibrin in the liver. Leukocytes (e.g., macrophages) are now recognized as important for liver repair after APAP overdose. We found that liver repair after APAP treatment was dramatically reduced in mice expressing a mutant fibrin(ogen) with normal clotting function but incapable of binding leukocyte αMβ2 integrin (Fibg390-396A mice). Decreased hepatocyte PCNA staining was observed in concert with increased hepatic congestion/necrosis in APAP-treated Fibg390-396A mice. Utilizing RNA sequencing, we found that the loss of fibrin(ogen)-leukocyte αMβ2 integrin binding inhibited APAP-mediated induction of genes associated with inflammatory processes, growth factors promoting regeneration, and extracellular matrix degradation. Among the genes with significant induction after APAP overdose was matrix metalloproteinase 12 (Mmp12), increasing to ~250-fold in APAP-treated wild-type (WT) mice, whereas Mmp12 increased to only ~25-fold in APAP-treated Fibg390-396A mice. Consistent with the concept that defective liver repair in Fibg390-396A mice was linked to diminished Mmp12 induction in these animals, we examined APAP hepatotoxicity in Mmp12-deficient mice, which developed increased congestion/necrosis and reduced hepatocyte proliferation relative to WT mice after APAP challenge. Collectively, these studies highlight an entirely novel pathway of liver repair after APAP overdose mediated by fibrin(ogen) engagement of leukocyte αMβ2 integrin and demonstrate for the first time a protective role of Mmp12 expression after APAP overdose.

1955 Evaluation of Nuclear Receptor-Mediated Alterations in Liver Cytochrome P450 Using Cryopreserved Rat Hepatocytes as a Screen for Potential Hepatocarcinogens

L. K. Markell, S. M. Waters, H. M. Peterson, D. L. Nabb and X. Han. *DuPont Haskell Global Centers, Newark, DE.*

Carcinogenicity bioassays are resource intensive, with a lengthy process for conducting the studies and interpreting results, in addition to the high cost and number of animals used in the study design. A positive finding may have a significant impact, yet the identification of a chemical as a human-relevant carcinogen may not occur until late stages of product development. Literature demonstrates that the constituitive androstane (CAR/CYP2B) and pregnane X (PXR/CYP3A) receptors do not have relevance to humans, while the arylhydrocarbon receptor (AhR/CYP1A) is assumed to be a relevant mode of action for human hepatocarcinogenesis. In order to proactively assess the potential for hepatocarcinogenesis and human relevance in longer-term studies, cryopreserved male Sprague Dawley rat hepatocytes were used to screen discovery chemicals for nuclear receptor-mediated alterations in liver cytochrome p450s. Cryopreserved hepatocytes were treated for 96 hours with eight discovery chemicals using 96-well collagen coated tissue culture plates and a matrigel overlay. Tested up to the limit of solubility, dose-dependent increases in 3A1 gene expression and testosterone 6β-hydroxylation activity, similar to that induced with the positive controls dexamethasone, was observed for all chemicals tested. However, pentoxyresorufin-O-deethylase (PROD) activity, indicative of CYP2B activity, was not detectable in the cryopreserved rat hepatocytes and the positive control phenobarbital did not induce CYP2B1 gene expression. Although induction of CYP1A1 gene expression and ethoxyresorufin O-deethylase (EROD) activity was observed, gene expression was 8-fold lower and enzyme activity 2-3 fold lower than the positive control β-naphthoflavone. For 3 of the chemicals tested *in vitro*, 14-day repeated dose studies were conducted. Correlating to results obtained in cryopreserved hepatocytes, induction of CYP3A1 gene expression, but not CYP1A1 was observed. These results demonstrate that cryopreserved rat hepatocytes may be used as a screening model for nuclear-receptor mediated activation of cytochrome p450s *in vivo*, providing mechanistic insight into modes of action relevant to hepatocarcinogens and aiding in product development decision-making.

1956 Evaluation of Perfluorooctanesulfonic Acid (PFOS) in a Model of High Fat Diet Feeding and High Fat to Low Fat Diet Switch

E. Martell, C. Picard, L. Armstrong, P. Shimpi and A. Slitt. *Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI.*

Non-alcoholic fatty liver disease (NAFLD) is characterized by increased fat content within the liver in the absence of alcohol use. NAFLD is most often related to obesity - with treatment being diet and exercise. Previous studies have shown that, in mice, PFOS administration increases liver weight and induces hepatic lipid accumulation. It was hypothesized that mice administered PFOS (0.003% in diet; ~360 μg/kg/day) would be slightly resistant to weight loss-induced improvement of NAFLD and more sensitive to high fat diet-induced hepatic steatosis. Mice were fed a standard chow low-fat diet (LFD) or 60% Kcal high-fat diet (HFD). At four weeks the mice were divided into two main groups - diet alone or diet containing 0.003% PFOS (~360 ug/kg/day). Then, for each group they were subdivided into 3 groups -mice fed LFD, mice fed HFD, or mice fed HFD that were switched to a LFD to mimic the therapeutic switch. This resulted in a total of 6 treatment groups as follows: i) LFD, ii) HFD-LFD, iii) HFD, iv) LFD + PFOS, v) HFD-LFD + PFOS, and vi) HFD+PFOS. For 10 weeks, mice were fed the new diets, and body weight and fasting blood glucose levels were monitored throughout the study. Serum triglycerides, non-esterified fatty acid, glucose, and total cholesterol levels were measured in fasting blood collected via submandibular bleed. A glucose tolerance test (GTT) and pyruvate tolerance test (PTT) were also administered to assess glucose utilization. PFOS exposure caused the reduction of body weight in the LFD group and less overall weight gain in LFD and HFD groups. PFOS exposure increased liver weight in the HFD and HFD-LFD groups and decreased WAT weight in the LFD group. The data suggests that PFOS exposure acts on lipid metabolism, lowering serum lipids, decreasing white adipose tissue mass, and increasing lipid content in the liver; thus, increasing the resistance to weight loss-induced improvement of NAFLD. Further studies on the tissues collected at necropsy will be utilized to confirm our physiological findings, and assist in determining the mechanism by which PFOS contributes to hepatic lipid accumulation.

1957 Effects of Vildagliptin and Its Metabolite on Inflammation-Associated Genes in Human Hepatoma HepG2 and Monocytic HL-60 Cells

M. Asakura, H. Fujii, K. Atsuda, T. Itoh and R. Fujiwara. *Graduate School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan.*

Vildagliptin is a potent, orally active inhibitor of dipeptidyl peptidase-4 (DPP-4) for the treatment of type 2 diabetes mellitus. Major metabolic pathway of vildagliptin is the DPP-4-mediated hydrolysis at the cyano group to produce a carboxylic acid metabolite M20.7. It has been reported that vildagliptin can cause hepatic dysfunction in patients. However, the molecular-mechanism of vildagliptin-induced liver dysfunction remains has not been elucidated. In this study, we determined hepatic genes that were highly regulated by vildagliptin in mice. We further examined the effects of vildagliptin and its metabolite on inflammation-associated genes, such as S100-calcium binding protein (S100) A8, S100A9, and tumor necrosis factor-alpha (TNF-alpha), in human hepatoma HepG2 and monocytic HL-60 cells. Mice were orally treated with vildagliptin (1000 mg/kg) and the livers were isolated 24 hours after the administration. The total RNA was prepared and the microarray analysis was carried out to determine the genes that were regulated by vildagliptin. Additionally, the expression levels of S100A8, S100A9, and TNF-alpha were determined in the control-, vildagliptin-, and M20.7-treated HepG2 and HL-60 cells. In the microarray analysis, we found that S100a8 and S100a9 were induced more than 5-fold by vildagliptin in the liver. In the HepG2 cells, vildagliptin, M20.7, and sitagliptin - another DPP-4 inhibitor - induced the expression of S100A9 and TNF-alpha. In the human monocytic HL-60 cells, in contrast, M20.7 specifically induced the expression of S100A8, S100A9, and TNF-alpha. The release of S100A8/A9 heterodimer in the cell culture media was observed in the HL-60 cells treated with vildagliptin and M20.7. These data indicate that not only vildagliptin, but also its metabolite M20.7 are involved in the onset of liver dysfunction. The M20.7-induced release of S100A8/A9 in monocytic cells might be a contributing factor of vildagliptin-associated liver dysfunction.

1958 Identification of Novel UPR Member IBTKα: Linking Autophagy to the Pathogenesis of Non-Alcoholic Steatohepatitis

J. A. Willy[2], S. K. Young[2], J. L. Stevens[2], H. C. Masuoka[2] and R. C. Wek[2]. [1]*Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN and* [2]*Department of Medicine, Indiana University School of Medicine, Indianapolis, IN.* Sponsor: J. Stevens.

Free fatty acid (FFA) induction of cell death is an important feature of non-alcoholic steatohepatitis (NASH) and has been associated with disruption of the endoplasmic reticulum and activation of the Unfolded Protein Response (UPR). However, the mechanisms by which this stress pathway results in deleterious effects at the cellular and organ levels are not well understood. In this study, we employ an *in vitro* model to evaluate the role of the UPR in NASH by treating HepG2 cells and primary human hepatocytes with saturated (palmitate and stearate) or unsaturated (oleate) FFA at physiologic concentrations. We show that saturated FFA, but not unsaturated FFA, induce hepatotoxicity through inhibition of autophagic flux as measured by accumulation of LC3b-II and P62. Saturated FFA are potent inducers of the UPR, including the transcription factor CHOP. Depletion of CHOP through CRISPR/Cas9 repressed induction of autophagy and enhanced cell viability following saturated FFA exposure. Conversely, addition of chloroquine or bafilomycin A1, inhibitors of lysosome activity, further enhanced LC3b and P62 accumulation in combination with saturated FFA-induced cell death. Together these results suggest that saturated FFA trigger cell death via a pathway involving UPR induction of autophagy, and the subsequent saturated FFA block in autophagy is critical for hepatocyte death. We are interested in the mechanisms by which CHOP and the UPR contribute to initiation of autophagy. We identified the α isoform of Inhibitor of Bruton's tyrosine kinase (IBTKα) as a member of the UPR. Expression of IBTKα is induced by the UPR by preferential translation and transcriptional induction through CHOP. Depletion of IBTKα through CRISPR/Cas9 resulted in increased cell survival and loss of phagaphore formation at the endoplasmic reticulum. To address the relationship between the UPR and inhibition of autophagic flux in NASH patients, we assayed liver biopsy samples and found up-regulation of CHOP and IBTKα coincident with accumulation of LC3b and P62 in NASH patients. Previous reports have suggested an association between the UPR and autophagy, and our study provides a mechanistic understanding of the roles the UPR and CHOP play in regulating saturated FFA induced hepatotoxicity at the cellular level.

1959 Inflammatory Response of Kupffer Cells in 3D Bioprinted Human Liver Tissues

R. N. Hardwick, J. Hampton, D. Perusse and D. G. Nguyen. *Tissue Applications, Organovo, Inc., San Diego, CA.*

Hepatic inflammation, mediated by Kupffer cells (KC), can exacerbate hepatocellular damage during drug-induced liver injury. KC are often employed in co-culture with hepatocytes to investigate the potential for inflammation in response to stimuli, such as the prototypical inducer lipopolysaccharide (LPS); however, such systems seldom include other nonparenchymal cells and fail to recapitulate the complex 3D interactions present in native liver. In the current study, LPS-mediated activation of KC at 24 and 72hrs was investigated in 3D bioprinted human liver tissues (exVive3D; Organovo, San Diego, CA). Induction of pro- and anti-inflammatory cytokines was measured via electrochemiluminescence in tissues comprising primary hepatocytes, stellate cells, and endothelial cells (Hep:KC-), and compared to tissues containing KC. Independent experiments were conducted comparing two KC donors (Hep:KC+D1 ♂ and Hep:KC+D2 ♀) with all other cell donors held constant. LPS stimulated TNF-α, IL-1β, IL-12p70, IL-10, IL-2, IL-13, and IL-4 levels in Hep:KC+D1 tissues at 24hrs compared to untreated, with sustained induction to 72hrs. TNF-α, IL-10, and IL-8 exhibited greater induction in Hep:KC+D1 tissues compared to Hep:KC-. All cytokines were increased at 24hrs in Hep:KC+D2 tissues treated with LPS compared to untreated, with sustained induction of IL-8, IL-1β, IFN-γ, IL-2, and IL-12p70. IL-8, IL-6, and IL-10 induction was greater in Hep:KC+D2 tissues compared to Hep:KC-. To compare donor-specific responses to LPS, cytokine levels were normalized to untreated Hep:KC-, and the fold change in LPS-treated Hep:KC+ tissues was calculated. Patterns of LPS-induced cytokine release were distinct between KC donors, with greater induction in the female donor. However, IL-1β, IL-10, IL-2, and IL-13 exhibited no variation in donor-specific response. The current data demonstrate the ability to measure a robust donor-specific KC response to inflammatory stimuli, thus enabling investigation of immune-mediated drug-induced liver injury in a 3D human liver tissue.

1960 Mouse Car is Mainly Involved in Liver Injury and Tumor Development Induced by the Protoporphyrinogen Oxidase Inhibitor Herbicide Acifluorfen

K. Kuwata[1,3,5], K. Inoue[1], M. Takahashi[1], R. Ichimura[1], T. Morikawa[1], Y. Kodama[2] and M. Yoshida[4]. [1]*Division of Pathology, National Institute of Health Sciences, Tokyo, Japan;* [2]*Division of Toxicology, National Institute of Health Sciences, Tokyo, Japan;* [3]*Drug Development Division, Mitsubishi Tanabe Pharma Corporation, Chiba, Japan;* [4]*Food Safety Commission of Japan, Tokyo, Japan and* [5]*Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo, Japan.*

Acifluorfen (ACI), a protoporphyrinogen oxidase (PROTOX) inhibitor herbicide, promoted the accumulation of porphyrin and induced tumors in rodent liver. Porphyria is accepted as a risk factor for liver tumors in rodents and humans; however, the precise mechanisms of PROTOX inhibition and porphyria have remained undetermined. We investigated the mode of action of ACI focusing on constitutive active/androstane receptor (CAR), which is essential for the development of rodent liver tumors in response to CYP2B inducers. Dietary treatment with ACI at 2500 ppm, an inducible dose for liver tumors in mice up to 13 weeks, increased Cyp2b10 expression in wild-type mice (WT), but the increase was blocked in CAR-knockout mice (CARKO). Hepatic Cyp4a10 expression indicating activation of PPARα was induced in both genotypes. ACI treatment induced cytotoxic changes in the liver including hepatocellular necrosis and inflammatory reaction. Concurrently, the number of PCNA-positive hepatocytes increased with prolongation of the treatment period in WT. In contrast, these hepatotoxic changes and the increase in PCNA positive cells were significantly and clearly attenuated in CARKO. ACI treatment led to increased liver protoporphyrin IX levels in both genotypes with comparable magnitudes, and the levels did not increase with a longer treatment period. No porphyrin deposition was found in hepatocytes from either genotype by microscopic or ultramicroscopic examinations. In the initiation-promotion model initiated by diethylnitrosamine, 26-week treatment with ACI at 2500 ppm clearly increased incidences and multiplicities of proliferative lesions including altered foci and adenomas of hepatocytes in WT, but these effects were much lower in CARKO. Regenerative hepatocellular hyperplasia and hepatocellular carcinoma were only detected in ACI-treated WT. Our present study suggests that CAR rather than porphyrin plays an important role in ACI-induced liver injury as well as tumor development.

1961 Intestinal HIF-1α Deletion Exacerbate Steatosis Signatures Associated with Gut Barrier Function and Microbiota

T. Shao[2], L. Zhang[2], L. Liu[2], F. Li[2], C. J. McClain[2] and W. Feng[2]. [1]*Division of Gastroenterology, Hepatology and Nutrition, University of Louisville, Louisville, KY and* [2]*Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Background: Alcoholic liver disease (ALD) is characterized by increased gut permeability and bacterial translocation. Hypoxia Induced Factor 1α (HIF-1α) has been implicated in transcriptional regulation of intestinal barrier integrity and inflammation. However, the link between intestinal HIF-1α regulation and ALD is unclear. We aimed to test our hypothesis that HIF-1α plays a critical role in gut microbiota homeostasis and the maintenance of intestinal barrier integrity in a mouse model of ALD. Methods: Wide type (WT) and intestinal-specific HIF-1α knockout mice (HIF-1α -/-) were pair-fed modified Lieber-DeCarli liquid diets containing 5% (w/v) alcohol or isocaloric maltose dextrin for 24 days. ALT and AST, endotoxin level, bacteria DNA and lipid panel were detected. Proinflammatory cytokines, liver triglyceride (TG) content and bacteria DNA were measured. Expression of intestinal HIF targets and proinflammatory cytokines were determined. Results: Alcohol feeding significantly increased serum levels of ALT, AST and LPS in the WT mice. These elevations were more pronounced in HIF1α -/- mice. Histology examination revealed that hepatic lipid accumulation is increased by alcohol exposure, and it was exaggerated in HIF1α -/- mice. Liver triglyceride concentration was increased by alcohol exposure in the WT mice and further elevated in the KO mice. Intestinal HIF-1α deletion resulted in a marked increase in hepatic inflammatory cytokine expression. In the HIF-1α -/- mice alcohol exposure disrupted intestinal structure integrity. 16s RNA analysis of the cecal and fecal samples of HIF1α-/- mice exposure to alcohol showed an elevated dysbiosis. Alcohol exposure decreased the expression of tight junction gene, occludin, and CRAMP, P-gp, and ITF, which are HIF1α transcription target genes and important for intestinal barrier integrity. Conclusions: Our results demonstrated that intestinal HIF1α is required for the adaptation response to alcohol exposure-induced changes in intestinal microbiota and barrier function leading to elevated endotoxemia and hepatic steatosis and injury.

1962 PCBs Diminish EGFR Signaling Pathways in Organopollutant-Mediated Steatohepatitis

J. E. Hardesty[3], K. C. Falkner[3], M. Cave[3], R. A. Prough[3], H. B. Clair[3] and B. Wahlang[1]. [1]*University of Kentucky, Lexington, KY;* [2]*Biochemistry and Molecular Genetics, University of Louisville, Louisville, KY and* [3]*School of Medicine, University of Louisville, Louisville, KY.*

Background: PCB exposure is linked to increased liver injury in human epidemiology studies and worsens liver injury in an obesity-driven murine model. PCBs are toxicants whose signaling is mediated by either the aryl hydrocarbon or constitutive androstane receptors (CAR). Phenobarbital has been shown to activate CAR by inhibiting Epidermal Growth Factor Receptor (EGFR) signaling. We hypothesize that PCBs may be indirect activators of CAR through inhibition of EGFR signaling and exacerbates steatoheptitis. Methods: Mice were fed either a control or high fat (HFD) diet and treated with either corn oil or Aroclor1260 (20 mg/kg) by gavage. Livers were removed for western blot analysis. *In vitro* cell culture assays were conducted with HepG2 and AML-12 cells. Cells were treated with EGF and either an EGFR inhibitor or Aroclor 1260 prior to western blot analysis. Results: Aroclor 1260 exposure robustly induced hepatic Cyp2b10 expression, but failed to induce Cyp1a2 expression *in vivo*. Aroclor 1260 failed to directly induce murine CAR reporters in transient transfection assays. EGFR expression was attenuated by HFD and Aroclor 1260 exposed mice had decreased phosphorylation of hepatic EGFR at Y1173, regardless of diet. PCB exposed mice had decreased EGFR signaling through the EGFR/PI3K/Akt/mTOR pathway and decreased STAT3 expression. Aroclor 1260 exposure was found to diminish EGFR phosphorylation at Y1173 in both the AML-12 and HepG2 cells in a congener-specific manner. Conclusion: These results demonstrate that PCBs inhibit EGFR-mediated signaling pathways, like the PI3K/Akt/mTOR pathway, a common feature of fatty liver diseases, explaining PCB action in steatohepatitis.

1963 Polymyxin B as a New Antidote Against Amanita Phalloides Poisoning: From *In Silico* to *In Vivo* Studies

J. Garcia[2], V. Costa[2], A. Carvalho[1], R. Silvestre[4], J. A. Duarte[2], D. Dourado[1], R. Dinis-Oliveira[2], P. Baptista[3], M. L. Bastos[2] and F. Carvalho[2]. [1]*Cell and Molecular Biology, Uppsala University, Uppsala, Sweden;* [2]*CIAFEL, Faculty of Sport, University of Porto, Porto, Portugal;* [3]*CIMO/School of Agriculture, Polytechnic Institute of Bragança, Bragança, Portugal and* [4]*ICVS/School of Health Sciences, University of Minho, Braga, Portugal;* [5]*UCIBIO - Laboratory of Toxicology, University of Porto, Porto, Portugal.*
Sponsor: S. Ali.

Amanita phalloides poisoning is the most common cause of dead resulting from mushroom poisoning (mortality >20%) worldwide. α-Amanitin is its main toxin and inhibits RNA polymerase II (RNAP II) causing hepatic and kidney failure. In the present study, we performed combined *in silico* and *in vivo* studies involving RNAP II for identification of potential antidotes against α-amanitin poisoning. The rationale was based on the putative bioisosterism of α-amanitin with the putative antidote. Docking and molecular dynamics simulation coupled to molecular mechanics-generalized born surface area method energy decomposition on RNAP II were performed to characterize the interactions between RNAP II and those drugs. The obtained results indicated that a clinically used drug, polymyxin B, potentially binds to the same interface as α-amanitin, which may prevent the toxin from binding to RNAP II. Subsequent *in vivo* studies in mice were done as a proof of concept of the *in silico* data. We demonstrated that a competition between α-amanitin and polymyxin B and/or displacement of α-amanitin from its RNAP II binding site by polymyxin B occurred, since multiple polymyxin B administrations [3 x 2.5 mg/kg intraperitoneal (i.p.)] 4 h after exposure to a lethal dose of α-amanitin (0.33 mg/kg, i.p.) significantly reverted the α-amanitin-induced transcription inhibition of renal specific genes, and decreased the α-amanitin induced liver and renal damage, 24 h after the toxins administration. During a survival rate assay, a 50% of survival of the α-amanitin-treated mice (until 30th day) in comparison with 100% of mortality by day 5 in animals exposed only to α-amanitin was observed. Moreover, a single dose of polymyxin B (2.5 mg/kg i.p.) administered concomitantly with α-amanitin was able to guaranty 100% survival until the 30th day post exposure. Taken together, these results show that polymyxin B protects RNAP II from inactivation leading to an effective prevention of organ damage and increase survival in α-amanitin-treated animals.

1964 Wnt/β-catenin Signaling Drives Thioacetamide-Mediated Heteroprotection Against Acetaminophen-Induced Lethal Liver Injury

V. P. Dadhania[2], B. Bhushan[1], U. M. Apte[1] and H. M. Mehendale[2]. [1]*Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS and* [2]*Toxicology, University of Louisiana at Monroe (ULM), Monroe, LA.*

Compensatory tissue repair (CTR) is a toxicodynamic process critical for the final outcome of chemical-induced tissue injury. Heteroprotection is a phenomenon where pre-placement of CTR in the form of compensatory liver regeneration in response to liver injury induced by a nonlethal dose of a toxicant affords protection against lethal dose of another toxicant. Whereas heteroprotection is a well-known phenomenon, the molecular mechanisms are not completely known. We investigated the mechanisms of heteroprotection using the thioacetamide (TA): Acetaminophen (APAP) heteroprotection model. Male Swiss Webster mice were treated with a low dose of TA (40 mg/kg, i.p.) 24 hr prior to a lethal dose of APAP (600 mg/kg, i.p., TA+APAP). Control mice were given distilled water 24 hr prior to APAP treatment (DW+APAP). Liver injury, tissue repair and the promitogenic signaling were studied over a time course of 0 to 24 hr post APAP treatment with or without TA pretreatment. TA pretreatment afforded 100% protection against the lethal dose of APAP, while DW+APAP group had 100% lethality. Whereas glutathione depletion was similar after APAP treatment in both TA+APAP and DW+APAP groups, a significantly lower activation of hepatic c-Jun N-terminal kinases (JNK) was observed in TA+APAP mice compared to DW+APAP mice. The TA+APAP treated mice exhibited extensive CTR characterized by significant cell proliferation, rapid and higher induction of core cell cycle proteins (cyclinD1 and cdk4) and increased phosphorylation of retinoblastoma (Rb). Robust liver regeneration in TA+APAP mice was secondary to the activation of Wnt/β-catenin pathway evident by the increased activated β-catenin and upregulation of its target genes, which was absent in the DW+APAP group. These data indicate that inhibition of JNK and rapid activation of Wnt/β-catenin signaling pathway drive TA-mediated heteroprotection of APAP-induced lethal liver injury.

1965 Fibrinogen Inhibits Biliary Hyperplasia and Fibrosis in Mice by Modulating IFNγ-Driven Macrophage Activation

N. Joshi[1,4], A. K. Kopec[1,4], H. C. Fedewa[4], C. E. Rockwell[1,4], M. J. Flick[2] and J. P. Luyendyk[1,4]. [1]*Institute of Integrative Toxicology, East Lansing, MI;* [2]*Div. of Exp. Hematology & Cancer Bio, Cincinnati Children's Hospital Medical Center, Cincinnati, OH;* [3]*Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI and* [4]*Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

Intrahepatic bile duct injury results in blood coagulation and deposition of fibrin in the liver. We found that xenobiotic (α-naphthylisothiocyanate, ANIT)-induced biliary hyperplasia and fibrosis were markedly increased in mice expressing a mutant fibrinogen that retains clotting function but is incapable of binding leukocyte αMβ2 integrin (Fibg390-396A). Here, we examined the possibility that fibrinogen mediates a response to inflammatory cytokines that reduces proinflammatory macrophage activation and fibrosis. Compared to ANIT-exposed wild-type (WT) mice, induction of Nos2 (M1 marker) was markedly increased in ANIT-exposed Fibg390-396A mice. In contrast, ANIT induction of Arg1 (M2 marker) was entirely blocked in Fibg390-396A mice. Notably, immune cell phenotyping confirmed an increase in intrahepatic M1-polarized proinflammatory macrophages in livers of ANIT-exposed Fibg390-396A mice. The capacity of surface-adhered fibrinogen (an αMβ2 ligand) to modulate cytokine-driven gene induction in cultured bone marrow-derived macrophages was also examined. Interestingly, fibrinogen suppressed IFNγ-mediated induction of Nos2, whereas it enhanced IL4-mediated induction of Arg1 expression. Under these conditions, other proinflammatory mediators (e.g. Tnf) were not affected, mirroring the selective differential gene expression in ANIT-exposed Fibg390-396A mice. Since Nos2-mediated nitric oxide production is linked to biliary hyperplasia and fibrosis, we hypothesized fibrin engagement of αMβ2 suppresses IFNγ-mediated Nos2 expression and resulting fibrosis in ANIT-exposed mice. Indeed, Nos2 expression and biliary fibrosis were significantly reduced in ANIT-exposed IFNγ knock-out mice. These results suggest that fibrinogen modulates xenobiotic-induced biliary fibrosis by cytokine-driven macrophage activation.

1966 SRT1720 Alleviates Cholestatic Injury in Mice by Altering Hepatic Bile Acid Composition and Enhancing Urinary Excretion of Bile Acids

S. R. Kulkarni[2], C. J. Soroka[2], L. R. Hagey[1] and J. L. Boyer[2]. [1]*Division of Gastroenterology, Department of Medicine, School of Medicine, University of California at San Diego, La Jolla, CA and* [2]*School of Medicine, Yale University, New Haven, CT.*

Sirtuin1 (SirT1, mammalian homolog of S. Cerevisiae enzyme Sir2) is a critical transcriptional and transactivational regulator of murine Fxr. Liver specific knockout of Sirt1 deregulates Fxr activity. Aim of this study was to determine whether cholestatic liver injury alters SirT1 expression and mechanisms by which Sirt1 activation may alleviate cholestatic liver injury. Relative to controls, hepatic SirT1 protein and mRNA expression was significantly downregulated in bile duct ligated (75%) and cholic acid (55%, 1%CA) fed mice accompanied by hyperacetylated Fxr and increased Jnk activation. To determine if SirT1 activator, SRT1720 reversed cholestatic liver injury, C57Bl/6 mice fed 1% CA for 5 days were concurrently administered vehicle or SRT1720 (50mg/kg/day, p.o) and liver injury markers, BA composition, gene and protein expression of BA metabolic genes and FXR acetylation were determined. SRT1720 administration in CA fed mice reduced plasma ALT (40%) and plasma BA (50%) levels while hepatic and total BA pool remained unchanged. Hepatic Jnk activated by CA feeding was reversed by SRT1720 administration restoring Sirt1 and Fxr mRNA, protein and acetylation levels to normal. SRT1720 significantly increased hepatic tetra- hydroxylated BA by 1.96x and decreased di-hydroxylated BA fraction to 21% over CA fed mice through induction of Cy2b10 (1.75x) mediated BA hydroxylation. SRT1720 inhibited hepatic BA synthesis via inhibiting hepatic Cyp7a1 (99%), 27a1 (75%) expression via induction of ileal Fgf15 (30x)-hepatic Fgfr4 (2x)- Shp mediated mechanisms. SRT1720 increased BA excretion in urine (2x) through induction of renal Mrp2 and Mrp4 expression (2 and 4.5x) relative to CA fed mice reflected by reduced plasma BA. Thus, SirT1 activators present a potential target for cholestatic liver injury.

1967 Synthetic Cathinones: Unveiling the Mechanisms for Liver Injury

M. d. L. Bastos[2], M. J. Valente[2], A. M. Araújo[2], F. Carvalho[2], P. Guedes de Pinho[2] and M. Carvalho[1]. [1]*FP-ENAS, CEBIMED, Fundação Ensino e Cultura Fernando Pessoa, Porto, Portugal and* [2]*Biological Sciences Department, UCIBIO-REQUIMTE, Laboratory of Toxicology, Porto, Portugal.* Sponsor: S. Ali.

Synthetic cathinones are a major group of new psychoactive substances which originally appeared as legal alternatives to amphetamines. From over seventy synthetic cathinones identified so far, 3,4-methylenedioxymethcathinone (methylone), 3,4-methylenedyoxypyrovalerone (MDPV), pentedrone and 4-methylethcathinone (4-MEC) are four of the most commonly used worldwide [1]. Several reports of intoxication and deaths related to the use of cathinones have been described over the past years [2; 3], but information on their toxicological properties is scarce. The purpose of this study was to evaluate the mechanisms underlying the hepatotoxicity of these four synthetic cathinones, at a low-effect and a worst-case approach concentrations (0.2 and 1.6 mM), in primary cultured rat hepatocytes. Cytotoxicity was determined by the MTT reduction assay. Production of reactive oxygen and nitrogen species (ROS and RNS), intracellular levels of reduced glutathione (GSH) and ATP, calcium homeostasis and caspase-3 activation were further evaluated to clarify the oxidative potential of each derivative and the mechanisms of cell death possibly involved. Data on MTT reduction showed that all four cathinones induced a significant decrease in cell viability after 48 h of incubation, and MDPV was found to be the most toxic one. All derivatives were able to induce the production of ROS and RNS at 1.6 mM, but only MDPV and pentedrone triggered both GSH and ATP depletion at this concentration. Similarly, only these two substances induced significant increase in calcium intracellular levels and activation of caspase-3. These data show that synthetic cathinones detain a great hepatotoxic potential, and oxidative stress plays a main role in cathinones-induced hepatic injury. However, the mechanisms underlying the cytotoxicity of each derivative may vary, so further studies are necessary to clarify this issue. [1] S. Elliott, J. Evans, Forensic Sci Int 243 (2014) 55-60. [2] J.M. Pearson, T.L. Hargraves, L.S. Hair, C.J. Massucci, C.C. Frazee, 3rd, U. Garg, B.R. Pietak, J Anal Toxicol 36 (2012) 444-51. [3] B.L. Murray, C.M. Murphy, M.C. Beuhler, J Med Toxicol 8 (2012) 69-75. This work was supported by FCT, through the project Pest-C/EQB/LA0006/2013.

1968 *Pterocarpus mildbraedii* Extract Ameliorates Propanil-Induced Hepatotoxicity by Abrogation of Oxidative Stress and Mitochondria-Mediated Apoptosis in Rats

C. A. Otuechere[2] and E. O. Farombi[1]. [1]*Drug Metabolism And Toxicology Research Laboratories, Department Of Biochemistry, University of Ibadan, Ibadan, Nigeria and* [2]*Division of Biochemistry, Department of Chemical Sciences, Redeemer's University, Ede Town, Nigeria.*

Liver diseases are reported to be associated with exposure to pesticides including propanil (PRP). The present study investigated the possible hepatoprotective potential of *Pterocarpus mildbraedii* extract (PME) against PRP induced oxidative stress and regulation of critical events in mitochondria mediated apoptosis. The four experimental groups evaluated included: Control, PRP (200mg/kg, po), PME (200mg/kg, po), and PME+ PRP for 7 days. The enzyme activities of serum alanine aminotransferase(ALT), aspartate aminotransferase(AST), alkaline phosphatase(ALP), lactate dehydrogenase(LDH) and gamma glutamyl transferase(GGT); hepatic superoxide dismutase, catalase, reduced glutathione, lipid peroxidation were determined spectrophotometrically. The protein levels of NFkB p65, phospho NFkB p65 and phospho IkBα were assessed using Enzyme-Linked Immunosorbent Assay. Key apoptotic markers such as caspase 3, caspase 9, Bax, and Bcl-2 were also observed in all the groups using immunohistochemistry to confirm involvement of the mitochondrial pathway. Histopathology of liver was determined by microscopy and apoptosis by TUNEL assay. The treatment with PRP caused significant elevation of serum AST, ALP, LDH, GGT activities, and MDA levels compared with controls. Levels of NFkB p65 were also lower in PRP treated rats. However, Caspase 9, Caspase 3, and Bax expressions were significantly reduced when PME was co-administered with PRP. Histology revealed severe hepatic periportal cellular infiltration in PRP treated rats. PRP also induced apoptosis by increased TUNEL assay positive nuclei. Our vivo findings regarding protection accorded by PME against propanil toxicity suggest that they reduced hepatotoxicity as well as attenuated critical control points of apoptotic cell death.

1969 Pparα and Car Receptors Differentially Regulate Hepatic Carboxylesterase Expression in Mice Treated with Perfluorooctanoic Acid

X. Wen[4], A. A. Baker[4], C. D. Klaassen[2], J. R. Richardson[3] and L. M. Aleksunes[1,4]. *[1]Environmental and Occupational Health Sciences Institute, Piscataway, NJ; [2]Dept of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA; [3]Dept of Pharmaceutical Sciences, Northeast Ohio Medical University, Rootstown, OH and [4]Dept of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

Perfluorooctanoic acid (PFOA) is a synthetic fluorochemical associated with hypercholesterolemia and hyperuricemia in exposed populations. In liver, PFOA can activate nuclear receptors including the peroxisome proliferator activated receptor alpha (Pparα) and constitutive androstane receptor (Car) and alter the metabolism and excretion of xenobiotics. Hepatic carboxylesterases (Ces) catalyze the metabolism of drugs, environmental toxicants and carcinogens. Previous studies demonstrated that Pparα and Car regulate the expression and function of Ces. The purpose of this study was to determine whether activation of Pparα and Car by PFOA could alter the expression of hepatic Ces. For this purpose, male wild-type (WT), Pparα-null, and Car-null mice were administered PFOA (1 or 3 mg/kg, po) for 7 days and livers were collected for assessment of Ces expression and activity. PFOA treatment increased the protein expression and activity of Ces1 2-fold in WT mice. Profiling of Ces mRNAs revealed that PFOA dose-dependently elevated levels of Ces1d, 1e, 1g and 2e between 1.5- and 2.5-fold, while it decreased Ces1c, 2b and 4a in WT mice. Activation of Pparα and Car by PFOA was confirmed by up-regulation of Cyp4a14a and 2b10 mRNAs, respectively. Pparα mediated the induction of Ces1c, 2b, and 3a genes by PFOA whereas Car mediated the repression of Ces1g expression. The differential regulation of Ces1d, 1e, and 2e genes by PFOA was independent of Pparα and Car signaling. In conclusion, the environmental contaminant PFOA modulates the expression of hepatic Ces, in part through Pparα and Car, which may be important for the metabolism of drugs and toxicants (Supported by R01ES020522, R01ES021800, T32ES007148, P30ES005022).

1970 Bile Canaliculi Dynamics Alterations as Early Preclinical Predictive Markers of Drug Induced Cholestasis

M. Burbank[2,3], A. Sharanek[2], A. Burban[2], N. Claude[3], R. J. Weaver[3], C. Guguen-Guillouzo[1,2] and A. Guillouzo[2]. *[1]Biopredic Int., Rennes, France; [2]INSERM U991, Rennes, France and [3]International Research Institute Servier, Paris, France.*

Drug-induced intrahepatic cholestasis remains largely unpredictable and mechanisms involved are still poorly understood. We recently observed that some cholestatic drugs caused early impairment of bile canaliculi (BC) dynamics typified by either constriction or dilatation of their lumen and associated with deregulation of the Rho-kinase/myosin light chain kinase (ROCK/MLCK) pathways (Sharanek et al. submitted). We aimed to evaluate the specificity of such BC dynamics alterations as predictive markers of drug-induced cholestasis using human differentiated HepaRG cells. BC dynamics were analyzed by phase-contrast imaging, and activity of ROCK/MLCK pathways and expression/activity of several canalicular and basolateral transporters were measured by appropriate techniques. A set of 17 drugs were tested; these were divided into 3 categories based on clinical observations: well-recognized cholestatic (5), very rarely cholestatic (6) and non-cholestatic (6). All cholestatic drugs but one (very rarely cholestatic) were correctly identified by phase-contrast imaging, causing either constriction or dilatation of BC after 2-3h of treatment. These early BC morphological alterations were associated with a decrease in BSEP activity with well-recognized cholestatic drugs and 2/6 very rarely cholestatic drugs. Accordingly, BSEP transcripts were also decreased after 24h with all cholestatic drugs, 4/6 rarely cholestatic drugs and one non-cholestatic drug. Interestingly, both ROCK and MLCK pathways, analyzed respectively by the myosin light chain phosphorylation level and by the use of a specific inhibitor, were found to be signaling targets of cholestatic compounds. In summary, our preliminary results suggest that analysis of alterations of BC dynamics associated with modulation of ROCK/MLCK pathways are promising predictive markers of drug-induced cholestasis. Support : EU Contract MIP-DILI-115336.

1971 Dual Role of Epidermal Growth Factor Receptor (EGFR) Signaling in Acetaminophen-Induced Liver Injury and Regeneration

B. Bhushan, M. W. Manley, Y. Xie, K. Du, M. R. McGill, M. Lebofsky, H. Jaeschke and U. Apte. *Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Epidermal Growth Factor Receptor (EGFR) signaling is known to play a crucial role in hepatocyte proliferation, but a few reports also suggest its role in cell death. Role of EGFR signaling in acetaminophen (APAP)-mediated liver toxicity and subsequent liver regeneration is completely unknown, which was investigated in this study. EGFR was remarkably activated as early as 30 min after APAP treatment in mice, in a dose-dependent manner and remained activated up to 24 hr. Interestingly, activated EGFR was rapidly translocated to mitochondria after APAP treatment. APAP-mediated glutathione depletion was found to be responsible for activation of EGFR. EGFR activation was also observed in primary human hepatocytes after APAP treatment, preceding elevation of toxicity markers. Treatment of mice with an EGFR inhibitor (EGFRI), Canertinib, 1 hr post-APAP caused robust inhibition of EGFR activation, resulting in striking reduction in APAP-induced liver injury. APAP-protein adducts formation and glutathione depletion were not altered indicating APAP metabolic activation was not affected by EGFRI. Further, APAP-mediated JNK activation (a key mediator of APAP toxicity) and its mitochondrial translocation were not altered by EGFRI. However, EGFRI treatment decreased APAP-mediated mitochondrial translocation of activated EGFR, nitrotyrosine protein adduct formation in mitochondria and blocked release of endonucleases from mitochondria, which are responsible for DNA damage and necrosis. Whereas, treatment with N-acetyl cysteine (conventional therapy for APAP overdose) 4 hr after APAP in mice did not show any protection, treatment with EGFRI, 4 hr post-APAP, showed remarkable decrease in liver injury. Interestingly, delayed treatment with EGFRI, 12 hr post-APAP, did not alter peak injury but caused remarkable impairment of liver regeneration response resulting in sustained injury and decreased survival after APAP overdose in mice. Impairment of liver regeneration was due to inhibition cyclin D1 induction and phosphorylation of retinoblastoma protein. In conclusion, our study has revealed an extremely novel role of EGFR both in development of APAP injury and in stimulation of subsequent compensatory liver regeneration after APAP overdose.

1972 Hepatotoxicity of Tyrosine Kinase Inhibitors

F. Paech, J. Bouitbir and S. Krähenbühl. *University Basel, Basel, Switzerland.* Sponsor: S. Messner.

Background: Tyrosine kinase inhibitors (TKI) are a new generation of chemotherapeutic agents. The first approved TKI was imatinib, initially for treatment of chronic myeloid leukemia. Many other TKIs have been approved for different indications since. Although TKIs are generally less toxic than the classical chemotherapeutic agents, they still have a narrow therapeutic window. Hepatotoxicity - mostly hepatocellular necrosis, but also cholestasis and hepatic cirrhosis - has been reported for several TKIs, including erlotinib, imatinib, lapatinib and sunitinib. The mechanisms underlying these effects still remain unclear. The aim of our study was to investigate the mechanisms of hepatotoxicity of erlotinib, imatinib, lapatinib and sunitinib *in vitro*. Methods: We treated human hepatoma cells (HepG2) with different concentrations of the 4 TKIs (between 1 and 100 M) for 6 to 48 hours. Results: Imatinib, lapatinib and sunitinib showed a time- and concentration-dependent cytotoxicity as well as a drop in intracellular ATP content in HepG2 cells. Reduced membrane potential in cells and isolated mitochondria, increased reactive oxygen species production, reduced GSH levels, reduced glycolytic flux, and induction of apoptosis was found for imatinib, lapatinib and sunitinib. Imatinib and sunitinib also showed a reduced maximal respiration with inhibition of complex III and I, respectively. Conclusions: Our investigations showed that imatinib and sunitinib lead to mitochondrial dysfunction whereas lapatinib seems to be a weak inhibitor of mitochondria. Furthermore, erlotinib is non-toxic for HepG2 cells for the tested conditions. As next steps we aim to determine susceptibility factors according to the proposed mechanism of liver toxicity and test them *in vitro* and *in vivo*.

1973 A High Cholesterol Diet Induces Mitochondrial Dysfuction and Liver Damage

M. Domínguez-Pérez[1], D. P. Rosales Cruz[1], N. Nuño Lambarri[1], M. Rosas Lemus[2], S. Uribe Carvajal[3], L. E. Gómez Quiroz[1], L. Bucio[1], V. Souza[1], R. U. Miranda Labra[1] and M. C. Gutierrez Ruiz[1]. *[1]Ciencias de la Salud, Universidad Autónoma Metropolitana, Mexico City, Mexico; [2]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico and [3]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico.*

Background: NAFLD is recognized as the most common form of liver disease worldwide affecting between 25 and 30% of the general population. Recent data suggest that disturbed hepatic cholesterol homeostasis and liver free cholesterol (FC) accumulation are relevant to the pathogenesis of NAFLD/NASH. Aim: To evaluate the effect of high chlesterol diet on mitochondrial functionality. Methods: C57/Bl6 mice were fed with regular chow diet supplemented or not with 2% cholesterol and 0.5% sodium cholate (HC) for 30 days. Liver tissue, hepatocytes and mitochondria were isolated for analysis of liver damage, lipid quantification and mitochondria morphology and function. Protein oxidation was addressed by OxyBlot™ kit. Mitochondrial structure was observed by transmission electronic microscopy (TEM). Mitochondria membrane potential was detected with safranine and confocal microscopy with MitoRed. Protein content was assayed by Western blot and caspase 3 activity and ATP content by spectrophotometric assay. Results: HC diet induced steatosis and increase in AST, ALT and the content of oxidized proteins. However, mitochondria presented a decrease in oxidized proteins. HC induced a differential expression in GPx isoforms, an increase in GPx-4 and a decrease in GPx-1. A decrease in mitochondrial size, but an increase in the number of them, were observed by TEM. Also, stress in endoplasmic reticulum was presented. Mitochondria membrane potential was decreased as well as caspase 3 activity and ATP content. p-Stat3 (Ser727) and its target genes Bcl-XL and Mcl-1, two anti-apoptotic proteins, were also increased as a result of HC diet. Conclusion: HC diet induced liver damage and dysfunction, associated with a decrease in mitochondrial membrane potential and ATP content. Mitochondrial protein oxidation was reduced, which could be due to GPx-4 increment. HC diet induced p-Stat3 (Ser727) and an increased content in Bcl-XL and Mcl-1, which could confer resistance to apoptosis. HC diet induced hepatocyte dysfunction associated with mitochondrial damage. Conacyt 166042 and PRODEP 913026-14612111

1974 Acetaminophen Induces Mitochondrial Oxidative Stress, Lipid Peroxidation, MIP1-a and MCP In HepaRG Cells

D. Thomas[3], M. Cottagiri[3], T. Stephens[3], J. Bonzo[4], M. Kumar[4], M. Powers[4], R. Witek[4] and D. Njoku[3]. *[1]ADME/Tox, Hepatic Biology, ThermoFisher, Frederick, MD; [2]Anesthesiology and Critical Care Medicine, Johns Hopkins, Baltimore, MD; [3]Biomedical Engineering and Cell Biology, Johns Hopkins, Baltimore, MD and [4]Cell Biology, ThermoFisher, Frederick, MD.* Sponsor: M. Powers.

Acetaminophen-induced liver injury is a primary cause for acute liver injury in patients. Freshly isolated hepatocytes have been utilized to study acetaminophen toxicity. Recently, HepaRG cells, an immortalized line of human cells that exhibit characteristics similar to primary hepatocytes and cholangiocytes by retaining drug metabolizing enzymes and receptors, were utilized to characterize oral acetaminophen toxicity. We hypothesized that HepaRG cells could elucidate roles for reactive oxygen species and chemokine expression by hepatocytes or cholangiocytes using intravenous acetaminophen. We treated HepaRG cells with the intravenous formulation of acetaminophen at 5mM, 10mM 15mM and 20mM for 2 hours and analyzed for mitochondrial oxidative stress with MitoSOX, for lipid peroxidase injury with Image-iT® and for glutathione with ThiolTracker fluorescent antibody stains. Chemokines were analyzed by Luminex® Assay. Maximum fluorescence intensity and cytokine levels were statistically analyzed using Mann-Whitney U test. A p value < 0.05 was considered significant. We found dose-dependent decreases in thiols ($p=0.0001$) while mitochondrial oxidative stress increased following treatments ($p<0.0001$). In sharp contrast, a biphasic response was demonstrated with lipid peroxidation where 10 mM treatments induced lower injury than 5 mM doses ($p<0.0001$) followed by significant dose-dependent increases thereafter. Granulocyte chemoattractants MIP-1β and MIP-1α were upregulated at 5 ($p<0.01$) and 20 mM($p<0.05$), respectively. Interestingly MCP-1 was downregulated at 5, 10 and 15 mM ($p<0.05$) doses. Previous studies have attributed chemokine expression to macrophage responses in acetaminophen toxicity; however, significant levels were detected using our model. We conclude that at lower doses of acetaminophen HepaRG cells exhibit signs of oxidative stress as well as chemokine expression critical for pro-inflammatory responses to injury. Interestingly, the monocyte chemoattractant MCP-1 is down-regulated at these same doses suggesting that acetaminophen hepatotoxicity may be more complex than previously imagined.

1975 Protective Effects of Carvacrol Against LPS-Induced Liver Injury

Z. Suntres. *Medical Sciences Division, Northern Ontario School of Medicine, Thunder Bay, ON, Canada.*

The purpose of this study was to evaluate the effectiveness of carvacrol in ameliorating the hepatotoxic effects of lipopolysaccharide (LPS). LPS, a major cell wall molecule of Gram-negative bacteria, causes liver injury *in vivo* that is dependent on neutrophils, platelets, and several inflammatory mediators. Carvacrol is a monoterpenic phenol produced by an abundant number of aromatic plants, including thyme and oregano. Carvacrol is used in low concentrations as a food flavoring ingredient and preservative; more recently, it has been shown that carvacrol possess a variety of biological properties including antioxidant, anti-inflammatory, and hepatoprotective. Sprague-Dawley rats were challenged with saline or LPS (Escherichia coli: 0111:B4; 1 mg/kg b.wt, iv); 4 hours later, animals were treated with saline, PEG 400, PEG 400/carvacrol solution (25 mg carvacrol/kg, ip) and killed 24 h post-LPS challenge. Liver injury was evaluated by measuring the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in plasma. LPS-induced activation of the inflammatory response was evaluated by measuring the levels of myeloperoxidase activity and chloramine concentration in liver homogenates as well as white blood cell, neutrophil and platelet numbers in blood. The hepatic levels of lipid peroxidation products and non-protein (NPSH) were used to assess the extent of involvement of oxidative stress mechanisms. In general, challenge of animals with LPS resulted in hepatic injury, activation of the inflammatory response, decreases in NPSH levels and increases in the levels of lipid peroxidation products (malondialdehyde and 4-hydroxyalkenals). Treatment of animals with carvacrol attenuated the LPS-induced liver injuries as evidenced by a general reversal of the altered biochemical indices toward normal levels among treated animals. These data suggest that carvacrol is a potentially effective agent in alleviating LPS-induced liver injuries. Funded by the Northern Ontario School of Medicine.

1976 Acrolein, a Lipid-Derived Aldehyde, Is a Pathogenic Mediator of Gut-Liver Injury in Alcoholic Liver Disease

W.-Y. Chen[3], J. Zhang[3], S. Barve[3], C. McClain[3] and S. Joshi-Barve[3]. *[1]Medicine, University of Louisville, Louisville, KY; [2]Pharmacology & Toxicology, University of Louisville, Louisville, KY and [3]Robley Rex VAMC, University of Louisville, Louisville, KY.*

Alcohol consumption can cause alcoholic liver disease (ALD), which remains a major cause of morbidity and mortality in the United States. Chronic alcohol consumption causes a pro-oxidant environment in the liver and increases hepatic lipid peroxidation. Acrolein (ACR) is the most reactive and toxic aldehyde generated through lipid peroxidation. ACR forms protein adducts and triggers endoplasmic reticulum (ER) stress and hepatocyte apoptosis, which are recognized etiologic factors in ALD. Several studies have established the critical role of the gut-liver axis in ALD pathogenesis, wherein alcohol-induced gut barrier dysfunction contributes to liver injury. This study investigates, *in vitro* and *in vivo*, the pathogenic role of acrolein as a major mediator of intestinal barrier dysfunction, and hepatic ER stress and injury in ALD. Accumulation of ACR adducts was seen in response to alcohol consumption in mouse livers and intestines. Intestinal ACR accumulation correlated with serum LPS, suggesting that elevated ACR is associated with gut permeability. Hepatic ACR buildup correlated with ER stress, steatosis, JNK activation, apoptosis and liver injury. Further, hallmark ER chaperones GRP78 and GRP94 were minimally induced, suggesting that ER-adaptive/protective responses were insufficient; the underlying cause was lack of activation of relevant transcription factors. We used cultured hepatic and intestinal cells to examine the direct *in vitro* effects of acrolein in comparison to alcohol. The *in vivo* gut-liver effects of alcohol consumption were mimicked by direct *in vitro* ACR exposure in intestinal Caco2 and hepatic H4IIEC cells. Specifically, ACR down-regulated tight junction proteins, altered their localization, and disrupted barrier function. Similarly, *in vitro* acrolein exposure in hepatic cells triggered ER stress and induced apoptosis. Notably, these alcohol-induced effects were attenuated by hydralazine, a known acrolein scavenger. Our study demonstrates that (i) acrolein is a mediator of alcohol-induced injury, and (ii) acrolein removal/clearance by scavengers has therapeutic potential in ALD.

1977 Role of DNA Damage Response in Liver Regeneration After Acetaminophen Overdose

P. Borude, B. Bhushan and U. Apte. *University of Kansas Medical Center, Kansas City, KS.*

Acetaminophen (APAP) overdose is a leading cause of Acute Liver Failure (ALF). Studies from our laboratory have shown that liver regeneration followed by APAP overdose plays a critical role in survival. A novel incremental dose model developed in our laboratory using a regenerating (300 mg/kg, APAP300) and a non-regenerating (600 mg/kg, APAP600) dose of APAP has revealed several pathways involved in regeneration after APAP overdose. Here we show that significant DNA damage leading to inhibited DNA repair results in prolonged activation of cell cycle checkpoint delaying liver regeneration. Liver injury, regeneration and microarray-based global gene expression changes were studied in male C57BL6 mice over time course of 0 to 72 hr following treatment with either APAP300 or APAP600. The ingenuity pathway analysis of microarray data revealed significant differences in DNA damage, replication and checkpoint related pathways between both doses of APAP. Western blot and immunofluorescence staining of pH2AX Ser139, a hallmark of DNA double strand break, showed prolonged phosphorylation of histone H2AX in mice treated with APAP600 than APAP300. DNA repair proteins involved in Non Homologous End Joining (NHEJ) pathway were significantly suppressed in APAP600, indicating delay or lack of DNA repair. Significantly high stabilization and activation of p53, a DDR effector protein, was observed in APAP600 as compared to APAP300. Studies in p53KO mice showed significantly higher injury than WT mice after APAP300 treatment at initial time points. Interestingly, despite the higher injury, p53KO mice recovered similarly as the WT mice due to faster cell cycle resulting in much higher regeneration. These data illustrate that lack of prompt DSB repair response after APAP overdose leads to p53-mediated prolonged growth arrest and replicative senescence. These results suggest that improving DNA repair process to rapidly decrease cell cycle checkpoint alert resulting in faster initiation of liver regeneration following APAP overdose may have therapeutic benefit.

1978 HGF Induces Liver and Lungs Protection Against Antituberculosis Drugs in a Model of Experimental Pulmonary Tuberculosis

O. Bello Monroy[1,2], C. Enriquez Cortina[2], O. A. Escobedo Calvario[2], U. Juárez Hernández[1], B. Ramos Robles[1], R. U. Miranda Labra[2], L. Bucio[2], V. Souza[2], D. Mata Espinosa[1], J. Barrios Payán[1], B. Marquina Castillo[1], M. C. Gutierrez Ruiz[2], R. Hernández Pando[1] and L. E. Gómez Quiroz[2]. *[1]INCMNSZ, Mexico City, Mexico and [2]Ciencias de la Salud, Universidad Autónoma Metropolitana, Mexico City, Mexico.*

Introduction: The increase in the number of cases of infections with multidrug resistant strains (MDR) of *Mycobacterium tuberculosis* is pointing out the necessity to find new therapeutics options. It has been proposed the elevation in the doses of drugs, such as rifampicin (RIF) or isoniazid (INH) could be useful for the elimination of the MDR bacteria, but liver can be compromised to damage. Hepatocytes growth factor (HGF) has been proved to induce protective effects in epithelial tissues. The aim of the present study was to address the effect of HGF in the treatment with high doses of RIF and INH in mice infected with a MDR strain of *Mycobacterium tuberculosis* and the impact in the bacterial load and liver damage. Methods: We used BALB/c mice that were infected with a MDR strain of *M. tuberculosis*. After mice have established the pulmonary progressive tuberculosis animals were treated with high doses or RIF and INH, using both, intratracheal or intragavage administration, and co-treated or not with HGF. Then mice were sacrificed at 30, 60 and 90 days. We determined colony formation units, and reactive oxygen species (ROS) by DHE fluorescence in lungs and liver, histology was assessed by routing H&E staining. Results: Data show that HGF treatment alone decreases bacteria in lungs, presenting a differential role in presence or absence of Mycobacteria, in addition the content of ROS decreased when mice were not infected and, increased under the infection, suggesting the activation of immunocompetent cells, this data correlated with the reduction in bacterial load and in the percentage of pneumonia, contributing to the lung healing. Hepatotoxicity of the liver due to drugs decreased significantly with HGF treatment. In Conclusion HGF could be considered as a good adjuvant in the treatment of tuberculosis due to the protective effect in the liver and lungs. Grant from Conacyt

1979 Troglitazone Inhibits Bile Acid Amidation: A Possible Risk Factor of Liver Injury

E. Ogimura, J. Deguchi, T. Nakagawa, M. Yabuki and H. Funabashi. *Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan.* Sponsor: T. Yamada.

Bile acid (BA) accumulation into hepatocytes is considered as one of the mechanisms of liver injury. It is known that non-amidated BA such as chenodeoxycholic acid (CDCA) shows more potent cytotoxicity compared with amidated BA such as glycochenodeoxycholic acid. Therefore, the inhibition of BA amidation by drug is assumed to be involved in liver injury. While inhibition of BA transporter is well known as one of the mechanisms of drug-induced liver injury, the inhibition of BA amidation is almost unclear. In this study, we investigated the intracellular BA concentration in hepatocytes treated with troglitazone (TRO) or pioglitazone (PIO) and the extent of amidated form to clarify the relationship between the inhibition of BA amidation and hepatotoxicity. Human hepatocytes were treated with 5 µmol/L CDCA or deoxycholic acid (DCA), one of the major BAs in human blood, in the presence/absence of 5 or 50 µmol/L TRO or PIO. Compared with vehicle control, intracellular accumulation of CDCA and DCA was observed in TRO treated group. In addition, the amount of their amidated form was decreased by TRO treatment. On the other hand, the amount of intracellular CDCA, DCA and their amidated form was decreased by PIO treatment. It was suggested that PIO could inhibit the uptake of CDCA and DCA into hepatocytes. Next, we examined the effect of TRO or PIO on BA amidation using human liver S9 fraction. TRO inhibited the amidation of CDCA and DCA. The IC50 values of TRO toward CDCA and DCA amidation were 5 and 3 µmol/L, respectively. On the other hand, PIO did not inhibit the amidation up to 100 µmol/L. From these results, it was suggested that the accumulation of non-amidated BA into hepatocytes caused by inhibition of BA amidation might be one of the mechanisms of TRO induced liver injury. To our knowledge, this is the first report of the inhibition of BA amidation by TRO.

1980 Use of Proliferating and Differentiated Upcyte Hepatocytes to Predict Toxicological Outcomes

A. Noerenberg[1], Y. Nahmias[3], G. Levy[3], S. Heinz[1] and A. Kark[2]. *[1]Upcyte Technologies GmbH, Hamburg, Germany; [2]Bio-Jump, Upcyte Technologies GmbH, Hillsborough, NJ and [3]Mircroliver Technologies Lab, The Hebrew University of Jerusalem, Jerusalem, Israel.* Sponsor: P. Hewitt.

Primary human hepatocytes have limitations for drug testing due to the low availability of human liver tissue. To address this, we have developed a novel technique which causes primary human hepatocytes to proliferate up to 40 population doublings producing 10^13 to 10^16 cells from a single human hepatocyte isolate whilst still retaining a metabolic competent polarized phenotype after oncostatin M removal in confluent cultures. The resulting cells are named "upcyte hepatocytes". To assess the utility of upcyte hepatocytes for toxicity screening, we used a library of known hepatotoxins and similarly structured control compounds. First, we compared the 24-h acute TC50 toxicity profile of six compounds in differentiated upcyte hepatocytes, primary human hepatocytes and HepG2 cells. A permutation test showed that the TC50 toxicity profile of differentiated hepatocytes for these six compounds was not significantly different from that of primary hepatocytes ($P = 0.466$; $n = 4$), whereas the HepG2 profiles were significantly different ($P = 0.03$; $n = 4$). We then measured TC50 values of 23 additional compounds in differentiated hepatocytes derived from four donors and found an excellent correlation between all donors of upcyte and primary human hepatocytes ($R^2 = 0.99$). Second, we compared the toxicological profile of proliferating and differentiated upcyte hepatocytes. The TC50 of nine known hepatotoxic drugs clustered into two groups. One group (aflatoxin B1, acetylsalicylic acid and acetaminophen) showed no difference between proliferating and differentiated cells. The second group, which included six of the nine hepatotoxic compounds, showed significantly higher toxicity in differentiated upcyte hepatocytes than in proliferating upcyte hepatocytes, suggesting that metabolic activation was required for the toxic effect. Our results demonstrate that expandable upcyte hepatocytes from multiple genotypes show toxicity profiles similar to those of primary human hepatocytes. Moreover, proliferating and differentiated upcyte Hepatocytes from the same donor can be used to discriminate mechanism of toxicity.

1981 Mitochondria-Targeted Antioxidant Protects Against Acetaminophen Hepatotoxicity

K. Du, J. Weemhoff and H. Jaeschke. *Department of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Background: Acetaminophen (APAP) hepatotoxicity is characterized by an extensive mitochondrial oxidant stress. However, its exact role in APAP induced-mitochondrial dysfunction and toxicity has not been well clarified. Purpose: Determine the role of mitochondrial oxidant stress in APAP induced-mitochondrial dysfunction and hepatotoxicity. Methods & Results: C57Bl/6J mice were treated with 300 mg/kg APAP, and mitochondria-targeted antioxidant Mito-Tempo (MT, 10 or 20 mg/kg) was given 1.5h later. Our data showed that APAP caused severe liver injury in mice at 3h and 6h, as indicated by the dramatic increase in plasma ALT activities and extensive centrilobular necrosis. MT dose-dependently reduced these two parameters. Importantly, this protection was not due to an effect on APAP metabolic activation since neither APAP protein adducts formation nor glutathione depletion was inhibited by MT. c-Jun N-Terminal kinase (JNK) activation or mitochondrial translocation was also not affected. In contrast, glutathione disulfide (GSSG) and peroxynitrite formation were dose dependently reduced by MT, indicating its effective mitochondrial ROS scavenging capacity. As a result, mitochondrial translocation of Bax and release of mitochondrial intermembrane proteins such as Smac and AIF were prevented. The corresponding nuclear DNA fragmentation was also largely reduced. To demonstrate the importance of mitochondria targeting property of MT, we compared its efficacy with Tempo, which has the same ROS scavenging capacity as MT but lacks the mitochondria targeting component. In contrast to the dramatic protection by MT, the same molar dose (6.1 mg/kg) of Tempo did not significantly reduce APAP hepatotoxicity. In contrast, the great protection by MT was up to 24h post-APAP, and even a 3h post-treatment reduced 60% of the injury, which is higher than the 38% reduction by the current clinical antidote N-acetylcysteine. Conclusion: MT protects against APAP overdose in mice by attenuating the mitochondrial oxidant stress and preventing peroxynitrite formation and the subsequent mitochondrial dysfunction. MT is a promising therapeutic agent for APAP-overdose patients.

1982 Inhibition of Mitochondrial Gene Expression by Deoxynucleoside Analogues and Delayed Mitochondrial Dysfunction

M. C. Korrapati[1], K. Toyokawa[1], B. Sherf[1] and J. Vanden Heuvel[1,2]. [1]*Indigo Biosciences, Inc., State College, PA and* [2]*Veterinary and Biomedical Sciences, Penn State University, University Park, PA.*

It has been recognized that many adverse effects associated with nucleoside reverse transcriptase inhibitors (NRTIs) used for the treatment of human immunodeficiency virus and hepatitis B virus are likely precipitated by mitochondrial toxicity. However, the attrition during clinical studies of ribonucleosides with anti-viral activity may be a reflection of inhibited mitochondrial RNA polymerase (POLRMT) and not direct alteration of electron transport or ATP production. Two deoxynucleoside analogues, 2'C methyl Adenosine (2'C MA) and 7-deaza Adenosine (7-dA) were examined for their ability to affect mitochondrial and nuclear gene expression, ATP production, oxygen consumption rate (OCR) and overt toxicity in the human hepatoma cell line Huh-7. After a 30 hr treatment with 2'C MA or 7-dA there was no significant effect on ATP production, OCR or toxicity. However, both nucleoside analogues decreased transcript levels of mitochondrial genes MT-CYB and MT-ND1, without affecting nuclear encoded NDFUA2 and UQCR2. These studies show the importance of evaluating mitochondrial transcript levels, at least with nucleoside analogues, when examining potential toxicity. Due to the high levels of mitochondrial DNA and relatively slow turnover of mitochondrial proteins, inhibition of POLRMT would not be captured in traditional mitochondrial function assays and may not be detected until clinical studies are performed.

1983 Understanding of Disease Mechanisms in NAFLD Using MS Imaging: Discovery of New Biomarkers Reflecting Disease State

G. Hamm[1], P.-M. Vaysse[1], A. van den Hoek[3], R. Kleemann[2], J. Stauber[1], H. M. Pincen[2] and D. Bonnel[1]. [1]*Imabiotech, Loos, France;* [2]*TNO, Leiden, Netherlands and* [3]*Metabolic Health Research, TNO, Leinden, Netherlands.*

The liver is the main organ of concern in drug discovery to assess efficacy or safety of a treatment. NAFLD (Non Alcoholic Fatty Liver) is emerging as a real heath corner stone in the progression from simple steatosis to more severe liver pathologies like cirrhosis. The characterization of this pivotal step using predictive biomarkers can improve the knowledge on the pathogenesis of the disease in various aspects such as inflammation, fibrosis, oxidative stress or modifications of lipid metabolism. As the hallmarks required for the diagnosis of NAFLD are still established on liver biopsy, the identification of biomarkers using MSI approach associated with histological staining has a very interesting role to play in this field. E3L.CETP and LDLR-/- mice fed with different diets were sacrificed and livers (12 tissues in each group) were removed. Mass spectrometric images were performed in positive or negative detection mode. Histological experiment involved H&E, Oil Red O and Masson's Trichrome staining. In this study, we investigated the targeted metabolite profiling in two animal models of metabolism disorders exposed to various diets to assess liver histological specificities. A multimodal approach to focus on various molecular classes (small metabolites & lipids) and using different polarities was used to describe liver modifications in NAFLD and NASH models. Potential disease or histological related biomarkers were observed especially at the level of lipids, phospholipids (PC) or lysophospholipids classes. Smaller molecules have been detected such as biliary acids near portal vein area or the GSH/GSSG couple. All these biomarkers were characterized on-tissue section using MS/MS experiments (SORI-CID mode) and high mass accuracy measurement (below ppm level). Potential correlation with fibrosis and inflammation process has been advanced at a biological point of view. Molecular distribution was correlated with H&E (for classical histology) and Oil Red O (for lipids droplets detection) staining on adjacent tissue section to highlight histological specificities related to metabolites levels. In conclusion, MSI was used to achieve a better understanding of underlying process occurring in liver disease development linking to metabolites changes.

1984 Critical Role of Mammalian Target of Rapamycin (mTOR) in Liver Damage Caused by VC Metabolites in Mice

A. L. Lang, B. R. Kaelin, H. Yeo, S. V. Hudson, L. G. Poole, C. M. McKenzie, C. N. Sharp, G. E. Arteel and J. I. Beier. *Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Background: Vinyl chloride (VC) is a prevalent environmental toxicant that has been shown to cause liver injury at high, occupational exposures. However, most studies have not addressed interactions of low doses with risk-modifying factors. We have shown that experimental VC/VC metabolites at doses that are not overtly hepatotoxic may sensitize the liver to hepatotoxicity caused by lipopolysaccharide. Moreover, these effects correlated with changes in hepatic energy metabolism. The kinase mTOR is a key regulator between pathways that control the balance of cellular energy metabolism and autophagy in response to stress. Therefore the role of mTOR was determined in this model. Methods: C57Bl/6J mice received chloroethanol (CE, 50 mg/kg, i.g.), a major VC metabolite, followed by rapamycin (1 mg/kg, i.p.) one hour later; 24 hours after CE, lipopolysaccharide was administered (LPS, 10 mg/kg, i.p.). Animals were sacrificed 24 hours after LPS administration. Plasma and livers were taken for determination of inflammation, histology, and liver damage. Results: Livers of control mice (vehicle, CE or CE + rapamycin) showed no signs of liver injury, inflammation, or increased cell death. LPS administration caused inflammatory liver injury characterized by a significant increase in plasma transaminase levels, pro-inflammatory markers (PAI-1, TNF-α), neutrophil accumulation as characterized by CAE histological staining, and necro-inflammatory foci. CE significantly enhanced all indices of liver damage and inflammation observed with LPS alone. However, the inhibition of mTOR by rapamycin significantly attenuated hepatic damage caused by LPS + CE. Transaminase levels were blunted, neutrophil infiltration was decreased, and inflammatory markers were inhibited. Conclusion: Taken together, these results suggest that mTOR is critical in mediating CE (as a surrogate for VC exposure) and LPS-induced liver injury. Previously, over activation of mTOR has been shown to cause an inhibition of autophagy resulting in increased liver damage. Therefore, the results here also support the hypothesis that autophagy may play a critical role in the development of liver injury due to VC exposure.

1985 Mechanism and Functional Consequence of MRP2 Mislocalization in Nonalcoholic Steatohepatitis

A. L. Dzierlenga, J. D. Clarke, D. M. Klein and N. J. Cherrington. *Pharmacology & Toxicology, University of Arizona, Tucson, AZ.*

The overlapping substrate specificity of hepatic multidrug resistance-associated (MRP) transporters, canalicular MRP2 and sinusoidal MRP3, provides a pertinent elimination pathway for a plethora of xenobiotics and metabolites. Disruption of this pathway contributes not only to the development of cholestasis, but to the interruption of proper biliary excretion of these compounds. MRP2 mislocalization from the canalicular membrane is proposed to occur via active endocytosis in periods of acute oxidative stress. Hepatic transporter misregulation has been well-documented in nonalcoholic steatohepatitis (NASH), the late stage of nonalcoholic fatty liver disease, characterized by fat accumulation, oxidative stress, inflammation and fibrosis. While an MRP2/Mrp2 mislocalization event is observed in both the human condition and the rodent methionine and choline-deficient (MCD) dietary model, the mechanism for this mislocalization remains unknown. The purpose of this study was to elucidate the mechanism of MRP2/Mrp2 mislocalization and the extent to which it impacts transport capability. To clarify the extent of impaired Mrp2 function in NASH, we compared the biliary excretion of a clinically relevant Mrp2 substrate in rats with MCD-induced NASH to rats deficient for Mrp2 (Mrp2-/-). We identified a transporter-dependent decrease in biliary excretion of 100% in Mrp2-/- rats and approximately 60% in the NASH group. To assess active endocytosis as a putative mechanism for MRP2 mislocalization, we measured the activation of various endocytic mediators implicated in MRP2 internalization *in vitro* by Western blot analysis. We have identified a decreased phosphorylation of the actin-binding protein radixin, as well as altered activation of protein kinases PKCα and PKA. Our data indicate that a perturbation of the endocytosis/exocytosis pathway may be a mechanism of Mrp2 mislocalization in NASH. Collectively, these studies increase the mechanistic understanding of MRP2 mislocalization in NASH, as disruption of the MRP2/MRP3 elimination pathway of xenobiotics contributes to increased systemic exposure and susceptibility to variable drug response.

1986 Evaluation of Respiratory Capacity Following Dili/Non-Dili Treatments; Seahorse Platform Optimization

D. Simic, M. Siegmann, L. Shanno, M. Singer and M. Otieno. *Preclinical Development & Safety, Janssen R&D (Johnson & Johnson), Spring House, PA.*

Drug Induced Liver Injury (DILI) remains a major reason for stopping drug development and withdrawal of a marketed drug. In general, there are two types of DILI, predictable (intrinsic) hallmarked by dose-response and predicted outcomes in preclinical species, and idiosyncratic DILI which are threshold based and difficult to predict in *in vivo* preclinical species. Regardless of the mechanism, intrinsic or idiosyncratic, it is becoming clear that mitochondria play a central role in DILI onset and/or outcome. By measuring the direct effect a compound has on the baseline oxidative phosphorylation (OXPHOS) of the cell, as well as, its effect on the maximal respiratory capacity of the mitochondria, Seahorse assay has become a powerful mechanistic tool for mitochondrial toxicity assessment. To correctly predict the mitochondrial (and DILI) risk, baseline assessment of 51 compounds (27 DILI and 24 non-DILI) for their ability to inhibit maximal respiration and/or cause uncoupling was performed. The acute (1h cell treatment) evaluation identified 12/27 (44%) of the DILI compounds as positive for mitochondrial toxicity (IC50 <50μM for mitochondrial OXPHOS inhibition or uncoupling), while no respiratory distress was noted with non-DILI compounds (IC50 >100μM). The compounds with IC50 <10μM were noted as potent inhibitors and included, Nefazodone, Troglitazone, and Benzarone. Treating cells for an extended period has reduced the number of false negative DILI compounds; it has shifted the IC50 and uncoupling concentrations, reflective of biologic significance and mitochondrial adaptability. Specifically, treating cells with amiodarone (iDILI mostly due to interference with fatty acids) for 1 and ≥6h shifted the IC50 from >100μM to ~10μM, respectively. While treatment with benzbromarone (reported hepatotoxicant) for 1 and ≥6h resulted in mitochondrial uncoupling at 3 and 30μM, respectively, emphasizing adaptability. In general, there were no difference between 6 and 24h treatments, indicating 6h time point as sufficient. Current efforts highlight the need to include at least a 6h treatment when using Seahorse platform in order to capture correct/biologically relevant IC50. Efforts to profile DILI compounds based on their mechanism of mitochondrial toxicity using Seahorse assay conditions will be outlined.

1987 Fasting-Induced Lipid Accumulation and Lipotoxicity in Hepatocyte-Specific Ppara Knockout Mice

C. Brocker, T. Yan, J. Bonzo and F. Gonzalez. *National Cancer Institute, National Institutes of Health, Bethesda, MD.*

Peroxisome proliferator-activated receptor alpha (PPARA) is a ligand-activated transcription factor and considered a key mediator of systemic lipid and carbohydrate metabolism. PPARA is activated in response to fasting and promotes the uptake, utilization, and catabolism of fatty acids by regulating peroxisomal, mitochondrial and cytochrome P450 enzymes involved in fatty acid oxidation. Studies have shown that PPARA has profound effects on fasting response and activation protects against fasting-induced oxidative stress. Fasted Ppara knockout mice (Ppara-/-) exhibit hepatic lipid accumulation and steatosis leading to elevated oxidative stress, lipid peroxidation and lipotoxicity. In order to elucidate the relationships between PPARA, fasting-induced hepatosteatosis, and oxidative stress, a hepatocyte-specific Ppara knockout mouse (PparaΔHep) was generated. Fasted PparaΔHep mice exhibit elevated serum phospholipids, cholesterol and triglycerides when compared to Ppara-/-. H&E and Oil red O staining indicated significantly less hepatic lipid accumulation when compared to full body knockouts. These data were supported by TBARS assays which revealed that lipid peroxidation was significantly lower in PparaΔHep mouse livers. ALT levels were elevated in both PparaΔHep and Ppara-/- mice, whereas, AST levels were significantly higher in Ppara-/- than PparaΔHep indicating increased hepatotoxicity. Unlike Ppara-/- mice, PparaΔHep mice do not display hypoglycemia in response to fasting. Furthermore, only Ppara-/- mice exhibited impaired glucose tolerance by intraperitoneal GTT. Alterations in glucose metabolism were supported by PAS staining which showed that impaired glycogen mobilization in Ppara-/- was dramatically reduced in PparaΔHep. Differential glycogen metabolism was further supported by qRT-PCR analysis of glycogenolytic genes which indicated slight but significant changes in rate-limiting enzymes. Taken together, these studies provide strong evidence that non-hepatocyte PPARA expression plays a major role in regulating lipid and glucose homeostasis during acute fasting response. Moreover, these studies serve as the foundation for future studies utilizing cell-type specific knockout mice to expand our current understanding of PPARA's role during physiological response to fasting.

1988 Three Dimensional Hepatotoxicity Screening Using Corning® HepatoCells, the Spheroid Microplate and the SCREEN-WELL® Hepatotoxicity Library

H. Sherman, H. Gitschier and D. Randle. *Corning Life Sciences, Kennebunk, ME.* Sponsor: C. Crespi.

Having the right model for drug screening is essential for predicting compounds that may cause drug-induced liver injury. Three dimensional (3D) models offer significant improvements over traditional two dimensional monolayer cell culture in terms of maintaining morphological and functional characteristics of tissue, and may provide a better representation of *in vitro* drug toxicity1. Here we demonstrate how Corning HepatoCells, an immortalized alternative to primary human hepatocytes, in conjunction with Corning Spheroid Microplates can be a utilized for a 3D drug screen to discover potential hepatotoxins. Hepatospheres formed using Corning HepatoCells were compared to spheroids formed using alternative immortalized hepatocyte cells. Cell viability, urea and albumin were used to assess hepatotoxicity of the hepatospheres after exposure to the SCREEN-WELL® Hepatotoxicity library from Enzo® Life Sciences, a library consisting of 238 compounds with a variety of structurally and mechanistically different compound classes, as well as nontoxic controls. Selected hits identified in the 3D screen, which significantly reduced cell viability of the hepatospheres, were then assessed in a dose-dependent manner for potency analysis. These results demonstrate that Corning HepatoCells, together with Corning Spheroid Microplates, are powerful tools that can be used for reliable and reproducible 3D hepatotoxicity screening.

1989 Development of a Co-Culture System of Endothelial and Hepatic Cells for Studying Busulfan-Induced Sinusoidal Obstruction Syndrome

K. Lidberg[2], T. Neumann[1] and E. J. Kelly[2]. [1]*Nortis Inc., Seattle, WA and* [2]*Pharmaceutics, University of Washington, Seattle, WA.*

Rationale: Busulfan is a front-line antineoplastic compound that has a high potential for causing the oft-fatal sinusoidal obstruction syndrome (SOS). Damage to the sinusoidal endothelium is thought to be a precipitating factor in disease progression; however, the underlying molecular mechanism(s) responsible for endothelial injury remains unknown. Elucidation of affected pathways essential for endothelial dysfunction seen *in vivo* has been hindered by a lack of toxicity of busulfan towards endothelial cells *in vitro*. We hypothesize that the deficiencies of *in vitro* models are due both to the type of cells tested as well as culture conditions. To test these hypotheses we are using endothelial colony-forming cells and a 3D microphysiological system (MPS), respectively. Methods: To determine optimal media composition for co-culture, morphology and proliferation of 2D ECFC and hepatic cell cultures were assessed in various growth media mixtures. Susceptibility of 2D endothelial-hepatic co-cultures to busulfan was evaluated using fluorescently tagged cytochrome C biosensors and real-time RT-PCR. A 3D MPS was used as a platform for developing a vascularized liver chip. Results: We report culturing conditions for ECFCs with HepG2 or HepaRGs in 2D as well as an optimized 3D MPS for long-term ECFC culture (>9 days). ECFCs are morphologically similar to human umbilical vein endothelial cells (HUVECs) but, when cultured with HepG2s form unique cord-like structures in 2D. Consistent with published data, HUVECs and ECFCs are insensitive to busulfan (100 μM-48 h) either alone or co-cultured with HepG2s, with comparable endothelial gene marker expression patterns. Discussion: We demonstrated ECFCs culture well with several hepatic lines as well as our 3D MPS and exhibit a unique phenotype with HepG2s. Given the highly specialized phenotype of the hepatic sinusoidal endothelium, the ability of ECFCs to be compatible with hepatic cells and display phenotypic plasticity makes them an optimal cell choice for studying SOS. Ongoing studies include establishment of an ECFC-vascularized MPS with various hepatic lines for evaluating busulfan-induced toxicities.

1990 *In Vitro* Hepatotoxicity Profiling Using Corning® Epic® Label-Free Technology with Primary Hepatocytes, HepG2 Cells, and Corning HepatoCells

A. B. Bergeron, H. J. Gitschier and D. H. Randle. *Corning Life Sciences, Kennebunk, ME*. Sponsor: C. Crespi.

To reduce time, expenses and resources in drug discovery, *in vitro* toxicity assays are used to detect adverse drug effects in earlier phases of drug development. In recent years, *in vitro* studies with primary human isolated hepatocytes have replaced animal models as the gold standard for liver toxicity studies, as they provide more accurate and less costly testing than animal models. However, primary human hepatocytes are available in limited supply and can exhibit variability between donor samples. Although immortalized cell lines, such as HepG2 cells, have been used to overcome these limitations, HepG2 cells display expression of drug-metabolizing enzymes that significantly differs from *in vivo*. Corning® HepatoCells are immortalized cryopreserved cells, derived from primary human hepatocytes that are consistent lot-to-lot and display primary hepatocyte characteristics, such as robust CYP3A4, 1A2 and 2B6 fold induction similar to primary hepatocytes. In conventional *in vitro* cytoxocity assays, specific cytotoxic indicators, such as ATP depletion, must be measured, but the mechanisms of chemical injury can be diverse and therefore several different indicators must be monitored. Additionally, the use of fluorescent or luminescent dyes, or radiolabeled compounds can introduce variables into the assay that are not replicated *in vivo*. Label-free dynamic mass redistribution (DMR) assays using Corning Epic Technology offer an alternative approach, capturing the cell signaling activity that occurs upon drug exposure in a single time-resolved measurement. In this study, primary hepatocytes, HepG2 cells, and HepatoCells were assayed with known hepatotoxins using the Corning Epic BT reader, demonstrating the ability of the technology to detect cytotoxic responses, and comparing the responses between the three cell types.

1991 A Comparison of *In Silico* Computational Programs for Predicting Drug-Induced Liver Injury

M. Masuda-Herrera and K. Ford. *Safety Assessment, Genentech, South San Francisco, CA.*

Drug-induced liver injury (DILI) has been a frequent cause of safety-related withdrawal of drugs from the market over the past 50 years. Hepatotoxicity is also a factor in the failure for continued drug development during research and development. Often, hepatotoxicity is not detected until later stages of drug development, at which point significant time and resources have been expended. *In silico* screening for hepatotoxicity provides a fast, high-throughput, and low-cost alternative to *in vitro* and *in vivo* testing without the need for test materials or animals. The purpose of this study was to evaluate and validate the ability of *in silico* models to predict hepatotoxicity. Initially, 143 marketed drugs were screened by *in vitro* methods with hepatocytes to classify compounds as either DILI or non-DILI. Of the 143 compounds, 80 were identified as DILI and 63 were identified as non-DILI. Next, a literature search was conducted to determine which compounds were hepatotoxic *in vivo*, of which 79 compounds were identified as DILI and 64 were identified as non-DILI. Finally, all 143 drugs were evaluated *in silico* using three different computational programs: Derek Nexus, Leadscope, and MultiCASE. In comparison to both *in vitro* methods and *in vivo* results, Leadscope provided the highest point estimate for correctly predicting both DILI and non-DILI compounds (71%). In addition, Leadscope had the highest point estimate for predicting true negatives (90%), whereas MultiCASE provided the highest point estimate for predicting true positives (71% compared to *in vitro* and 75% compared to *in vivo*). Statistically, combined *in silico* methods achieve comparable specificity and/or sensitivity as a single-method approach, but tend to be on the more conservative side for predicting hepatotoxicity. The results from this study support the use of *in silico* models as a first-line screening for predicting hepatotoxicity for drugs in development because they provide fast, high-throughput analysis with high concordance to *in vitro* methods and *in vivo* results.

1992 A Multispecies Comparison of the Cytotoxicity of Riddelliine in Primary Hepatocytes

W. Resager, B. Stegelmeier and S. Colegate. *US Department of Agriculture, Agricultural Research Service, Poisonous Plant Research Laboratory, Logan, UT.*

Dehydropyrrolizidine alkaloids (DHPAs) and their N-oxides are a potentially hepatotoxic, pneumotoxic, genotoxic and carcinogenic. DHPA-producing plants are commonly weedy by nature and, hence, can contaminate food supplies for both livestock and humans, the latter resulting in potentially fatal hepatic veno-occlusive disease. A growing concern is the potential link between intermittent or long-term, low-level dietary exposure of humans to DHPAs and chronically-developing diseases (eg cancers, pulmonary arterial hypertension, congenital abnormalities). Approximately 500 different DHPAs, comprising monoesters, open chain diesters and macrocyclic diesters of a few core necine bases, have been identified and toxicity is structure- and target species-dependent. Direct comparisons of DHPA toxicity are also difficult as published works have used different animal models, exposure routes and durations. To better predict risk, comparative studies evaluating purified alkaloid toxicity using identical models, routes and molarity doses are needed. DHPA structural differences, including stereochemical variations, can hinder large-scale isolation of many of these compounds needed for *in vivo* studies. Therefore, an *in vitro* primary hepatocyte model was developed to assess the 50% inhibitory concentration (IC50) of riddelliine (the model DHPA used in this comparison). Because of their extensive use in *in vivo* models of toxicity, mice, rats and chickens were selected for this initial comparative primary heptocytotoxicity model. Viability of the hepatocytes was determined, using a MTT mitochondrial activity assay, after a 24-hour exposure period to riddelliine in concentrations ranging from 100 nM to 1.2 mM. Chicken hepatocytes were shown to be the most susceptible, with preliminary assessment indicating rat and mouse hepatocytes being at least 60 and 120 times more resistant respectively. The *in vitro* IC50s for the mouse, rat and chicken primary hepatocytes reflected the broad range of *in vivo* susceptibility between the species reported in the literature. Therefore, with low requirements for amounts of pure alkaloids for testing, this primary hepatocyte model offers the potential to directly compare the hepatocytotoxicity of various DHPAs in the most susceptible primary hepatocyte line, and for comparison between species.

1993 Epalrestat Causes Apparent Apoptosis in Cultured Mouse and Human Hepatoma Cells

L. Chen and X. Cheng. *Department of Pharmaceutical Sciences, St. John's University, Queens, NY.*

Epalrestat (EPS), an aldose reductase inhibitor (ARI), is prescribed to treat diabetic neuropathy in Japan and Europe. Epidemiology studies showed that hepatic dysfunction is the most common adverse reaction reported in EPS therapy. The risk of causing liver injury limits its therapeutic applications. Currently, the underlying mechanism by which EPS produces liver injury remains largely unknown. Therefore, the present study was designed to investigate the modes of action by which EPS produced liver injury. MTT cell proliferation assays showed that in cultured mouse Hepa1c1c7 and human Hep3B hepatoma cells, EPS decreased cell viability in a dose-dependent manner. 200μM of EPS decreased cell number by more than 80% in both cell lines. In addition, immunocytochemistry staining assays showed that EPS dose-dependently increased protein level of cleaved Caspase-3 in both cell lines, indicating that EPS decreased cell number possibly mediated by enhanced apoptosis. Furthermore, we showed that EPS increased mRNA expression of heme oxygenase (HO)-1 and NADPH: quinone oxidoreductase (Nqo)-1 in both Hepa1c1c7 and Hep3B cells, indicating that nuclear factor erythroid-derived 2-related factor 2 (Nrf2) signaling is activated by EPS. In summary, our study demonstrated that EPS exhibits toxicity in both mouse Hepa1c17 and human Hep3B hepatoma cells possibly via enhanced apoptosis, which may compensatorily activate Nrf2.

1994 Development of a Translational High-Throughput Assay for Drug Induced Liver Injury in Zebrafish Larvae

A. D. Vliegenthart[1], C. Wei[1], J. Del Pozo[1], D. J. Antoine[2], P. Treskes[1], D. J. Webb[1] and J. W. Dear[1]. *[1]University of Edinburgh, Edinburgh, United Kingdom and [2]University of Liverpool, Liverpool, United Kingdom.*

Zebrafish have important advantages over rodents for mechanistic studies of drug-induced liver injury (DILI). They are cheap and easy to house, zebrafish are a model organism that is high throughput with a functionally similar liver and innate immune system to humans, their transparency as embryos allows easy cell tracking and drug administration is straightforward. Triptolide (TP) is the main active ingredient purified from the plant *Tripterygium wilfordii* and has been used for centuries in traditional Chinese medicine. However the clinical use of TP is restricted due to a high incidence of hepatotoxicity. The objective of this study was to develop a TP induced liver injury model in zebrafish larvae. The zebrafish larvae were exposed to TP dissolved in system water for up to 48 hours (3-5pdf). Hepatotoxicity was assessed using multiple modalities including: mortality, histology, liver RNA (determined by PCR and RNA seq) and time lapse microscopy performed on agar imbedded larvae from the liver fluorescent Tg(LFABP:GFP) zebrafish line. Histologically, TP induced hepatocyte swelling and vacuolation from 2 hours after the start of drug exposure and hepatocyte disarray from 6 hours after the start of exposure. Triptolide induced a dose-dependent mortality and reduction in copy numbers per fish of the liver specific microRNA-122. Triptolide induced hepatotoxicity could be faithfully tracked in fish by measuring fluorescent intensity. RNA seq identified multiple toxicity pathways that mediate TP induced liver injury. In conclusion, TP is a potent hepatotoxic drug that can be modelled in zebrafish larvae and time-lapse experiments in combination with a liver specific fluorescent reporter can track TP induced liver toxicity in real time. The zebrafish is a valuable model organism for studying DILI that can bridge from *in vitro* models to rodents.

1995 Human Hepatocyte/Kupffer Cell 3D Spheroid Co-Cultures: Characterization and Application for DILI Studies

P. Gunness[2] and A. P. Li[1]. *[1]In Vitro ADMET Laboratories LLC, Columbia, MD and [2]In Vitro ADMET Laboratories LLC, Malden, MA.*

The goal of the study was to develop procedures to reproducibly culture hepatocytes as spheroids and to characterize long-term 3D human hepatocyte spheroid co-cultures for their suitability for DILI studies. Multiple lots of cryopreserved human hepatocytes were evaluated for their ability to be cultured as spheroids using hanging drop plates (1000 cells per culture, 96-well plate format). Only plateable cryopreserved human hepatocytes were found to be successful in the formation of spheroids. Spheroid formation was highly reproducible from these lots (5 out of 5 independent cultures). Hepatocyte/Kupffer cell co-cultured spheroids were produced and maintained for 4-6 weeks in culture. The spheroids were characterized at days 7, 14, 21, 28, 35, and 42 for morphology, hepatic functions and response to hepatotoxicants. The spheroid cultures maintained viability (based on cellular ATP contents) and a consistent size (300-350 μm) over the culture period. Histological evaluation of H&E stained formaldehyde fixed spheroids showed intact, cuboidal hepatocytes with tight cell-cell junctions and without a necrotic core. The long-term cultured spheroids were found to maintain hepatic functions including human albumin synthesis (quantified in culture medium using ELISA) and cytochrome P450 3A4 activity (luciferin IPA metabolism). LPS treatment of the cultures led to induction of the proinflammatory cytokine IL-6. Dose- and treatment duration-dependent cytotoxicity was observed with model hepatotoxicants acetaminophen, troglitazone, tolcapone and aflatoxin B1. Results show that hepatic spheroids can be reproducibly prepared from cryopreserved human hepatocytes with stable hepatic functions upon prolonged cultures. Responsiveness of the human hepatic spheroids to hepatotoxicants at relevant concentrations and prolonged exposure periods suggest that this experimental model may be used for the evaluation of drug hepatotoxicity at conditions resembling human exposure *in vivo*, thereby may allow a more accurate prediction of DILI potential of the drugs in question.

1996 Modeling Drug-Induced Hepatic Fibrosis *In Vitro* Using Three-Dimensional Liver Tissue Constructs

L. M. Norona[2,3], D. G. Nguyen[1], D. A. Gerber[3], S. C. Presnell[1] and E. L. LeCluyse[2,3]. *[1]Organovo, Inc., San Diego, CA; [2]The Hamner Institutes, Research Triangle Park, NC and [3]The University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Compound-induced hepatotoxicity leading to fibrosis remains a challenge for human toxicity risk assessment. Latency to detection and lack of early biomarkers make it difficult to characterize the dynamic and complex intercellular interactions that occur during progressive liver injury. Animal models only partially address this challenge however; the development of a truly mechanistic understanding requires the use of human tissue-like model systems that can be interrogated over time to identify key cellular and molecular events underlying fibrogenesis. Here we demonstrate the utility of bioprinted tissue (exVive3D Human Liver, Organovo) comprising hepatocytes, stellate cells and endothelial cells to conduct repeated low concentration modeling of compound-induced liver injury leading to fibrosis. Significant time dependent elevations of LDH were observed for both TAA (by Day 3) and MTX (by Day 11) and were accompanied by the acquisition of a fibrogenic phenotype as supported by preliminary cytokine, gene expression data, and histologic evidence of collagen deposition. In comparison, treatment with TGF-β1, a known pro-fibrogenic cytokine, yielded moderate to severe fibrotic change in the tissue with little evidence of hepatocellular damage. These preliminary data provide strong proof-of-concept that 3D bioprinted liver tissues can recapitulate drug-, chemical- and TGF-β1-induced fibrogenesis on a cellular, molecular, and histological basis. The use of this novel system and strategy will enable the integration of initial and adaptive cellular mechanisms during the onset and progression of injury to better characterize key attributes unique to fibrogenic agents useful for improving chemical risk assessment.

1997 Functional Characterization of Human Induced Pluripotent Stem Cell-Derived Hepatocytes Differentiated at Flow Conditions Using Human Precision-Cut Liver Slices as Benchmark

V. Starokozhko[3], M. Hemmingsen[2], L. Larsen[2], S. Mohanty[2], M. Merema[3], R. Pimentel[2], A. Wolff[2], J. Emnéus[2], A. Aspegren[1], G. M. Groothuis[3] and M. Dufva[2]. *[1]Cellartis, Takara Bio Europe AB, Göteborg, Sweden; [2]Department of Micro- and Nanotechnology, Technical University of Denmark, Lyngby, Denmark and [3]Groningen Research Institute of Pharmacy, University of Groningen, Groningen, Netherlands.* Sponsor: F. G. Russel.

Human induced pluripotent stem cell (hiPSC)-derived hepatocytes are recognized as an alternative cell source for bioartifical liver (BAL) development. However, the hepatic differentiation of hiPSCs under flow conditions in a scaffold has never been described before. Moreover, the evaluation of the differentiation of the generated hepatocytes using primary human hepatocytes (PHH) as a benchmark is hampered by the loss of function of PHH during culture. Therefore, the aims of this study were to induce hepatic differentiation of hiPSCs under flow conditions and perform functional characterization of the hepatocytes using fresh human precision-cut liver slices (hPCLSs). The expression of genes responsible for liver development (HNF4α, CK7, BGP), synthesis (ALB), metabolism (CYP3A4, CYP3A5, CYP2B6, CYP3A7, CAR) and transport (PGP, BSEP) was studied, as well as Phase I and Phase II (sulfation

and glucuronidation) metabolism activities, and albumin, urea and bile acid production. We have shown that the majority of the tested CYP activities, phase II metabolism, and albumin, urea and bile acid synthesis in the BAL system reached values that overlap with those of the hPCLS. Only CYP2B6 activity was lower in the BAL than in hPCLS. Gene expression data indicates that iPSCs differentiated into both hepatic and biliary directions, as epithelial biliary cell markers were well expressed in cell cultures. In conclusion, the BAL developed under flow conditions reflects several liver functions at levels comparable to hPCLS and shows excellent perspectives to be further developed to a BAL system used for toxicity testing or as a liver-support device for patients in the clinic.

PS 1998 Evaluating Reactive Acyl Glucuronides Formation from Diclofenac Using a Resazurin/ Resorufin Assay with Primary Rat Hepatocytes

N. Hari Singh[1], M. McMillian[1], Y. Qu[1], C. W. Ng[3], Y. Zhou[3], H. Yu[3] and A. Ananthanarayanan[1]. *[1]Invitrocue Pte Ltd, Singapore, Singapore; [2]Centre for Mechanobiology, National University of Singapore, Singapore, Singapore and [3]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.* Sponsor: M. Johnson.

Previously we reported that increased resazurin reduction to resorufin and/or decreased removal of resorufin in response to carboxylated drug candidates is consistent with reactive acyl glucuronides (RAGs) generated from parent drugs in HepG2 cells (Hari Singh et al, 2015). NQO1 appears to be the critical enzyme inhibited by RAGs in HepG2 cells, resulting in inhibition of resorufin fluorescence removal, with the increased fluorescence providing an easy, convenient screen for evaluating compounds for RAG activity (Hari Singh et al, 2015). However, HepG2 cells are metabolism deficient and although enough glucuronidation is present to detect RAGs, the sensitivity of the resazurin assay is low. In the present study, we have taken advantage of the much higher metabolic capacity (glucuronidation and Cyp activities) in freshly isolated rat hepatocyte cultures, to examine the sensitivities of three NSAIDs in the RAG resazurin assay. Diclofenac, diflunisal and flufenamic acid all produced RAGs at concentrations similar or far below their therapeutic concentrations. At low concentrations, diclofenac (unlike the other two NSAIDs examined) is well known to be metabolized by Cyps rather than glucuronidated. Inhibition of Cyp 2C by sulfaphenazole (Kretz-Rommel and Boelsterli, 1993) produces a robust increase in sensitivity of the RAG resazurin assay to diclofenac, suggesting more parent drug becomes glucuronidated at low concentrations after Cyp inhibition. The sensitivity of the assay to diflunisal and flufenamic acid were not similarly increased by Cyp inhibitors. The RAG resazurin assay in rat hepatocytes shows extreme sensitivity to these NSAIDs (diclofenac, diflunisal and flufenamic acid) and may help identify compounds early in Drug Development where Cyp metabolism is more important (like diclofenac) than glucuronidation at low concentrations.

PS 1999 3D Organotypic Liver Models for High-Throughput Hepatotoxicity Testing

S. M. Orbach[2], M. F. Ehrich[2] and P. Rajagopalan[2]. *[1]Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA and [2]Department of Chemical Engineering, Virginia Tech, Blacksburg, VA.*

There are currently thousands of chemicals whose effects on human populations are unknown. Even those chemicals that have been identified as hazardous to health are often not fully characterized with respect to molecular targets, initiating events, and long-term health effects. High throughput screening (HTS) can begin to bridge this vast gap in our knowledge. Traditionally, HTS assays have been conducted with monolayers or 2D cell cultures. Such studies, while useful, do not provide holistic information at the multi-cellular or tissue levels. We have assembled a novel liver organotypic culture model (OCM) assembled in 96-well plates (denoted henceforth as µOCM) that will enable the simultaneous measurements of the effect of individual and cocktails of chemicals. A novel aspect is the introduction of automated procedures to assemble the µOCMs. An automatic cell dispenser was used to seed primary rat hepatocytes, liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) in a 96-well plate. A polymeric Space of Disse separated hepatocytes from LSECs and KCs in the 3D models. Acetaminophen (APAP) and troglitazone were administered on day 4 for 24 h. Hepatocyte monolayers (HM) and collagen sandwiches (CS) served as 2D comparisons. HM cultures maintained greater than 88% viability even when treated with 40mM APAP and 200 µM troglitazone. CS cultures only demonstrated a significant, 32 ± 14% decrease in viability at 200 µM troglitazone. The 3D models exhibited up to 71 ± 4% and 62 ± 4% cell death when treated with APAP and troglitazone, respectively. Ongoing efforts are focused on the use of HTS assays to investigate changes in hepatic cellular functions, protein expression and functional markers in the presence of individual and cocktails of chemicals. By seamlessly combining HTS methodologies with a liver µOCM we seek to provide a platform for medium to high throughput testing of toxicants.

PS 2000 In-Depth Characterization of 3D Human Liver Microtissues by Transcriptomic, Proteomic and Functional Analysis

S. Messner[2], L. Fredriksson Puigvert[4], C. Escher[1], M. Bober[1], K. Roessger[2], J. M. Kelm[2], W. Moritz[2] and M. Ingelman-Sundberg[4]. *[1]Biognosys AG, Schlieren, Switzerland; [2]InSphero AG, Schlieren, Switzerland; [3]Karolinska Institutet, Stockholm, Sweden and [4]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.*

3-dimensional (3D) spheroid cultures of primary human hepatocytes were previously shown to prolong viability and liver-specific functionality in comparison to 2D cultures. However, little is known about how donor-to-donor differences of primary human hepatocytes are reflected in 3D liver microtissues. Here, we provide a thorough in-depth characterization of 3D human liver microtissues derived from 3 different human hepatocyte donors in co-culture with primary liver Kupffer cells over 5 weeks. The 3D liver microtissues were analysed for viability, morphology, basal cytochrome activity, cytochrome inducibility, transcriptomic and proteomic profile over the whole culture period. The 3D liver microtissues exhibited stable ATP-content and albumin secretion over 5 weeks in culture. Histological sections indicated presence of a healthy tissue with high glycogen incorporation. Bile canaliculi were established and exemplified by BSEP expression. Distinct donor variability was seen for CYP 1A2, 3A4, 2B6, 2C8, 2C9, 2D6 and 2C19 activity. For example, CYP2B6 and CYP2D6 showed little activity in 1 of the 3 donors. However, the basal cytochrome activity remained stable for most of the CYPs over the whole culture period. Inducibility of CYPs was tested with Omeprazole, Phenobarbital and Rifampicin induction of 3D liver microtissues, which showed >2 fold induction of the corresponding CYPs even after 4 weeks of culture. Transcriptomic analysis by whole genome arrays revealed distinct similarities to native human liver gene expression, despite high inter-donor differences in liver specific gene expression. Proteomic profiling of more than 3500 proteins over the whole culture period using HRM-MS Technology showed that only around 350 proteins were up- or downregulated more than 2-fold from day 7 to day 35 in culture, suggesting that the 3D human liver microtissues are very stable over time. In summary, this characterization exemplifies that 3D human liver microtissues are a robust organotypic liver model and is therefore suitable for various applications in drug metabolism and toxicity testing.

PS 2001 Functional Evaluation of Bioprinted Human Liver Tissue as a Liver Injury Model

K. Tetsuka[1], M. Ohbuchi[1], H. Moriguchi[1], E. Kobayashi[2], M. Kanki[2], A. Miyashita[1] and K. Tabata[1]. *[1]Analysis & Pharmacokinetics Research Labs., Astellas Pharma Inc., Tsukuba-shi, Japan and [2]Drug Safety Research Labs., Astellas Pharma Inc., Osaka-shi, Japan.* Sponsor: A. Okada.

Purpose: As drug-induced liver injury is a major cause of drug withdrawal from market, culture models that can accurately predict and evaluate the toxic potential of chemicals are required. Three-dimensional (3D) bioprinting technology enables the alignment of different cell types in a spatially-controlled manner. Here, we characterized the long- and short-term effects of acetaminophen (APAP) in a 3D-bioprinted model of the human liver. Materials and Methods: The exVive3D Liver (Organovo Holdings Inc., San Diego, CA, USA) was used as a three-dimensional human liver tissue model (3DHLT). Briefly, 3DHLTs were constructed from human hepatocytes, stellate cells, and endothelial cells of the umbilical vein using a Novogen Bioprinter (Organovo Holdings Inc.). To measure the long-term effects of APAP, 3DHLTs underwent treatment with APAP at 0, 0.3, 1, 3, 10, or 30 mM for 1, 7, 14, or 28 days. To measure the short-term effects of APAP, 3DHLTs underwent treatment with APAP at 30 mM for 6 h, followed by either termination of culture or further culture without APAP for 1, 3, or 18 h as a washout stage. Intracellular ATP and glutathione (GSH) levels in these specimens were then measured as described in the manufacturer's instructions. Results and Discussion: Treatment of 3DHLTs with APAP at 30 mM for 1 day significantly reduced mean ATP level to 27.9% of that of controls. Prolonged APAP treatment also reduced mean ATP levels, even with treatment at lower concentrations. IC50 values decreased over the APAP treatment period. Short-term APAP treatment of 3DHLTs at 30 mM for 6 h significantly reduced mean GSH level to 56% of that of controls but did not affect mean

ATP level. Compared to controls, GSH levels in subsequent cultures of 3DHLTs without APAP were as follows: 46% (1 h), 72% (3 h), and 101% (18 h). These results suggest that GSH levels recover following washout of APAP, implying the biologically viable nature of 3DHLTs in culture. In conclusion, the 3DHLT is applicable in evaluating hepatotoxicity caused by long-term compound treatment, as well as liver recovery following the removal of chemicals.

2002 A High Throughput Imaging-Based 3D Spheroid HepG2 Toxicity Pathway Reporter Platform for Repeated Dose Toxicity Evaluation

S. Hiemstra, S. Wink and B. van de Water. *Toxicology, Leiden University, Leiden, Netherlands.*

Adaptive stress response activity is a measurement for early cellular perturbations after chemical exposure. In order to quantitatively assess the adaptive stress response activation and dynamics we developed an *in vitro* screening platform using HepG2 cells and BAC-GFP transgenomics technology. Here we evaluated the application of our BAC-GFP reporter cell lines for repeated dose toxicity assessment in differentiated 3D HepG2 spheroids. HepG2 cells cultured in 3D matrigel stop proliferating and demonstrate strong differentiation. In this study, HepG2 cells stably expressing GFP tagged stress response proteins were cultured as 3D spheroids. Characterization of the differentiation status of individual reporter lines using immunofluorescence for bile duct formation as well as drug metabolism capacity for key cytochrome P450, indicated that all reporter cell lines differentiated equally. We then established an automated intelligent imaging pipeline for confocal imaging-based reporter activity assessment. To further assess reporter activation in 3D spheroids, we selected six reporter cell lines representing three different adaptive stress response pathways (oxidative stress: Srxn1-GFP, NQO1-GFP; unfolded protein response: BiP-GFP, Chop-GFP; and DNA damage: p21-GFP and Btg2-GFP). All reporter cell lines were screened with chemicals known to induce activation of these genes (based on TG-GATEs toxicogenomics data). We determined the response after 48h exposure as well as after six and eleven days daily repeated exposures. Positive reporter responsive were observed for the various conditions, with six day repeated exposure being most optimal. Subsequently, we selected a larger subset of 31 known DILI compounds, which were screened for both 48h single dosing and six day daily repeated dosing. Overall our data indicate that 3D spheroid HepG2 GFP stress response reporters are an improved model that contain liver-like properties and allow repeated dose exposure studies for mode-of-action assessment of novel drug candidates.

2003 Utilization of exVive 3D Human Liver Tissues for the Evaluation of Valproic Acid-Induced Liver Injury

C. Grundy, R. Smith, J. Nickel, R. N. Hardwick and D. G. Nguyen. *Tissue Applications, Organovo, Inc., San Diego, CA; Tissue Testing, Organovo, Inc., San Diego, CA.* Sponsor: D. Nguyen.

Conventional (2D) cell culture models do not accurately reflect the complex microenvironment of liver tissue, and pre-clinical animal trials are often inadequate due to species-specific variation in hepatocellular functions. 3D bioprinted human liver tissues better approximate human tissue composition and physiology, and therefore enable the assessment of drug-induced liver injury (DILI) and related mechanisms at the tissue level, including biochemical and histologic outcomes. In this study, we assessed the DILI response to Valproic Acid (VPA), a compound known to induce steatosis in humans, in 3D-bioprinted human liver tissue mimetics comprised of primary hepatocytes, hepatic stellate cells, and endothelial cells. 3D-bioprinted human liver tissues (exVive3D; Organovo, San Diego CA), were treated daily for 14 days with VPA, which resulted in dose-dependent decreases in tissue health as assessed by ATP, GSH and histology. Tissue ATP levels were decreased 70% and 45% relative to vehicle following 14 day treatment with 1mM and 5mM VPA, respectively. Following the observations seen with ATP levels, the higher doses of 200µm, 1mM and 5mM were selected to further evaluate the mechanism of observed tissue damage. GSH levels were measured at 24hr and 72hr to determine the acute oxidative stress response. A significant decrease in the ratio of reduced to oxidized GSH was observed at 24hr treatment, indicating increased oxidative stress. Recovery of GSH ratios was noted with 200µm and 1mM treatment groups at 72hr, while the 5mM treatment group exhibited prolonged oxidative stress. Histological evaluation of tissues revealed dose-dependent damage with abundant vacuolization in 5mM VPA treated tissues, consistent with a steatotic phenotype. Collectively, these data suggest exVive 3D human liver tissues can be used to investigate the mechanisms of DILI *in vitro*, as evidenced by the expected results seen in both biochemical and histologic endpoints following treatment with VPA.

2004 Novel Integrated *In Vitro* Gastrointestinal and Hepatic Model for Investigating Chemical Toxicity

R. Less and P. Rajagopalan. *Department of Chemical Engineering, Virginia Tech, Blacksburg, VA; School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, VA.* Sponsor: M. Ehrich.

To date, *in vitro* models of both the liver and the gastrointestinal (GI) tract have been developed individually with very limited efforts to integrate them into a single system. These models provide limited information on the complementary actions of the GI and liver. There is a surprising lack of *in vitro* models that can simulate how the liver and GI complement each other's role *in vivo*. In an attempt to bridge this gap, our goal is to design and assemble an *in vitro* model of the GI tract and liver using either Caco-2 cells or a GI sheet integrated with a 3D multi-cellular organotypic liver model. Towards this goal, we first integrated a monolayer of Caco-2 cells with primary rat hepatocytes cultured in a collagen sandwich. Acetaminophen (APAP), a prototypic hepatotoxicant, was administered over a 5-20mM concentration range. When 5mM of APAP was administered, Caco-2 cells exhibited 24±1.3% decrease in their trans-epithelial electrical resistance only in the integrated model. This loss in the gap junction integrity of Caco-2 cells was accompanied by a 41±8.0% decrease in albumin production, a 59±10% decrease in urea production and an AST/ALT ratio of 1.62±0.27 in hepatocytes. The decrease in hepatocyte function was attributed to the generation of reactive oxygen species in Caco-2 cells upon APAP administration. Interestingly, loss in gap junction integrity was not observed in Caco-2 cells cultured individually. Similarly, hepatocytes cultured individually did not exhibit markers of toxicity until a high concentration of APAP was administered. Ongoing efforts are focused on integrating a GI cell sheet with a 3D multi-cellular organotypic liver model. The changes in hepatocyte function such as functional markers and expression of cytochrome P450 proteins will be monitored. Additionally, changes to the structure of the GI cell sheet are also being investigated. Recreating the organ-to-organ interaction between the GI and the liver will serve as a powerful platform to systematically decipher the effects of drugs and chemicals.

2005 Validation of a High Content Imaging Assay for Steatosis and Phospholipidosis in a Micropatterned Human Hepatocyte Co-Culture Model

C. Deisenroth, K. Wolf, J. Trask, E. LeCluyse, R. Clewell and M. Andersen. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Non-alcoholic fatty liver disease (NAFLD) is a serious health concern affecting an estimated 20% of the U.S. population. Altered lipid homeostasis in the liver, as either steatosis or phospholipidosis, represents major key events for progression to liver injury or diseases such as NAFLD. While components of the metabolic syndrome (obesity, type II diabetes, dyslipidemia, and insulin resistance) are primary contributors to fatty liver disease, certain environmental chemical exposures have been linked to lipid accumulation in the liver. These are proposed to work through a number of modes-of-action (MOA) including mitochondrial impairment, enhanced cytokine production, and altered hepatic lipid secretion. To establish a human relevant *in vitro* model for predicting chemical effects on fatty liver disease, a multiplexed high-content screening assay using a 96-well micropatterned co-culture system of primary human hepatocytes was developed to assess the contribution of prioritized chemical exposures to phenotypic endpoints of steatosis and phospholipidosis. Five reference drug compounds were selected for assay development due to the high specificity and well understood MOAs: cyclosporin A and methotrexate (steatosis), propranolol (phospholipidosis), amiodarone (phospholipidosis and steatosis), and caffeine (negative control). Optimization of dosing parameters and kinetics resulted in a 96 hour workflow with a fixed endpoint, 5-channel assay to quantitatively determine hepatocyte-specific neutral lipid accumulation, phospholipidosis, cell counts, and viability. High-content imaging parameters for lipid endpoints were restricted to analyzing lipid droplet size, intensity, and frequency. Anticipated concentration-response curves for the lipid endpoints were observed for all of the compounds except for methotrexate. In addition, cytotoxicty parameters were validated and performed as expected. The results demonstrate a suitable *in vitro* platform fit for the purpose of evaluating chemical effects on human fatty liver disease.

2006 Primary Hepatocytes under Hemodynamics and Transport Conditions Mimic Toxicity Signatures to Amiodarone and Propylthiouracil in the Clinic

R. Figler, S. Marukian, M. S. Collado, M. Lawson, A. Mackey, D. Manka, B. Blackman, B. Wamhoff and A. Dash. *HemoShear Therapeutics, Charlottesville, VA.*

Drug induced liver injury (DILI), a major cause of pre- and post-approval failure, is challenging to predict pre-clinically. While animal models may fail to capture species-specific responses, human *in vitro* hepatocyte cultures typically require 50-100 times the maximal plasma drug concentrations, limiting the ability to understand toxic mechanism pathways at exposures that mimic the clinical setting. We previously described a system that uses hepatic sinusoidal flow parameters to restore primary hepatocyte biology and drug responses at clinical plasma concentrations. Amiodarone (AMD) and propylthiouracil (PTU) have distinct clinical and pathological DILI profiles resulting in a steatohepatitic/fibrotic and hepatocellular phenotypes respectively. We assessed these drugs at a concentration approximating clinical therapeutic exposure (Cavg) and a toxic concentration (10x Cmax) within the system. Primary human hepatocytes (n=5 donors) were exposed to AMD (2.3 μM and 39 μM) or PTU (20.4 μM and 393.6 μM) for 48 hours. Unbiased transcriptomic analysis by RNAseq yielded distinct biological pathway themes around mitochondrial respiration, cholesterol biosynthesis, extracellular matrix (ECM) organization, coagulation and inflammation for both drugs. Pathway analysis revealed upregulation of fatty acid and cholesterol biosynthesis with suppression of lipid metabolism by AMD, predictive of steatohepatitis, and confirmed functionally by increased intracellular nile red (lipid) staining in hepatocytes. Fatty acid oxidation and mitochondrial respiration were activated in PTU with suppression of triglyceride and cholesterol synthesis, and no evidence of lipid accumulation. The fibrotic potential of AMD was evidenced by activation of TGF-β signaling and ECM remodeling genes, unlike PTU. Antigen presentation and inflammation genes were suppressed by PTU, suggesting a possible immunologic response. The correlation of these cellular responses to clinical effects reported with the drugs demonstrates the system's ability to predict distinct toxicity signatures at clinically relevant concentrations and differentiate mechanisms of DILI.

2007 A Human Heart-Liver Platform to Study Drug Metabolism and Toxicity

C. Oleaga[3], G. Legters[3], L. Kumanchik[3], V. Platt[3], L. R. Bridges[3], C. Martin[3], M. Jackson[3], C. W. McAleer[3], C. J. Long[3], J. Langer[2], A. Riu[1], R. Note[1], J. J. Hickman[3], S. Teissier[1], J. Cotovio[1] and L. Breton[1]. *[1]L'Oreal Research and Innovation division, Aulnay-sous-Bois, France; [2]L'Oreal Research and Innovation division, Clark, NJ and [3]NanoScience Technology Center, University of Central Florida, Orlando, FL.* Sponsor: E. Dufour.

The regulatory context of March 2013 requires efforts to strengthen the development of alternative methods, in the field of cosmetic ingredients, to assess systemic toxicity. However, conventional *in vitro* assays do not fully represent physiological conditions. Xenobiotic metabolism is the missing piece of the puzzle in non-hepatic toxicology assays. Thus, we suggest connecting the metabolic engine of the liver to an organ susceptible of xenobiotic damage for the generation of an *in vitro* toxicity system to bridge the gap that exists or for physiologic toxicity prediction. We have developed a human heart-liver system to study drug metabolism dependent toxicity. This platform combines cardiac and hepatic modules that maintain full functionality in serum-free medium, under flow, for 14 days. Upon drug treatment, changes in (i) cardiac beating frequency and contractile force, and (ii) hepatic p450 activities, albumin and urea productions were studied. The drugs, cyclophosphamide and terfenadine, used for the validation of this system, represent both case scenarios; a non-cardiotoxic pre-drug that converts into a cardiotoxic metabolite, and the opposite, a cardiotoxic pre-drug that converts into a non-cardiotoxic metabolite. It is commonly accepted that the integration of a metabolic function in future toxicology models can improve adverse effects prediction in humans. Our system integrates this concept and enables a functional readout of cardio- and hepatotoxicity under serum-free conditions for acute and chronic exposure of drugs and their metabolites. The next steps will consist of better characterizing the model and screening a larger set of reference compounds.

2008 A Promising, *In Vitro* Approach to Classify Drugs According to Their Potential to Cause Idiosyncratic, Drug-Induced Liver Injury

A. Maiuri, B. Wassink, J. Turkus, A. Breier, T. Lansdell, G. Kaur, P. Ganey and R. Roth. *Michigan State University, East Lansing, MI.*

Idiosyncratic, drug-induced liver injury (IDILI) occurs in a small fraction of susceptible patients and can be life threatening. Importantly, current methods employed during preclinical safety evaluation of drug candidates fail to identify those with IDILI liability before they reach the market. Accordingly, assays to identify drug candidates with the potential to cause IDILI early in the drug development process are needed. Evidence in humans and animals suggests a role for immune mediators in the pathogenesis. Some drugs that cause IDILI stress hepatocytes, rendering them susceptible to killing by cytokines. In this study we tested the hypothesis that cytotoxic synergy between drugs and inflammatory cytokines can accurately classify drugs according to their IDILI liability. Of a set of 14 drugs associated with human IDILI, almost all synergized with tumor necrosis factor-alpha (TNF) and interferon gamma (IFN) to cause injury (LDH release) to HepG2 cells *in vitro*, whereas 10 drugs with little or no IDILI liability demonstrated no such synergy. Detailed concentration-response relationships were determined for calculation of parameters such as the maximal LDH release, slope and EC50. These and other parameters were used singly or in combination as covariates to construct several best-fit logistical regression models. For some of these models, receiver operating characteristic analysis revealed an excellent ability to classify the 24 drugs correctly, with areas under the curve (AUCs) >0.95 and small 95% confidence intervals. These results suggest a highly promising *in vitro* approach employing a single, phenotypically relevant endpoint (cell death) that is amenable to high throughput methodology and that could be used preclinically to identify drug candidates with the potential to cause IDILI in human patients. (Supported in part by a Colgate-Palmolive Grant for Alternative Research from the SOT).

2009 High Content Screening of Corning® HepatoCells for Hepatotoxicity

H. Sherman, H. Gitschier and D. Randle. *Corning Life Sciences, Kennebunk, ME.* Sponsor: C. Crespi.

Revealing compounds that have a negative impact on the liver early in a drug development campaign is important in drug discovery. Drug induced liver injury is one of the most cited causes of drug candidate failure, and is often the reason for withdrawal of approved drugs from the market1. Therefore, having the correct model system and assay for screening is extremely important for detecting possible hepatotoxins. Corning HepatoCells are an immortalized alternative to primary human hepatocytes, that still retain the metabolic and enzymatic functionality of primary human hepatocytes, but without the lot-to-lot variability and reliance on human donors. Here we demonstrate how Corning HepatoCells can be a utilized to discover hepatotoxins by way of multiparameter high content screening analyses. Specifically, lipid accumulation and mitochondrial membrane potential loss were examined after exposure to a variety of known hepatotoxic compounds. High content analysis of hepatotoxicity using Corning HepatoCells plated in Corning BioCoat Collagen I High Content Imaging Microplates, was compared to that of alternative immortalized hepatocyte cells. These results indicate that Corning HepatoCells, together with Corning High Content Imaging Microplates, are powerful tools for reliable and reproducible *in vitro* hepatotoxicity screening to predict liver injury.

2010 Detection of Drug Cholestatic Potential Using Confocal High Content Screening in HepaRG and Human Liver Microtissues

P. Walker, S. Ravenscroft and C. Dilworth. *Cyprotex, Cheshire, United Kingdom.* Sponsor: C. Strock.

Drug-induced cholestasis is a frequently observed side effect of drugs and often results from an impaired bile acid export leading to intrahepatic accumulation. Bile acid transport and metabolism is essential in maintaining bile acid homeostasis. BSEP (bile salt export pump) is the key membrane transporter responsible for the transport of bile acids from hepatocytes into bile. As there are known difference in transporters in human and rodents, we developed two human microtissue (MT) models to investigate pro-cholestatic compound detection. HepaRG and human liver MTs (hLiMTs) create a 3D cellular environment thus facilitating bile canaliculi formation and the expression of BSEP and MRP2 (multidrug resistance associated protein 2) on the apical membrane towards the bile canaliculi. It was shown that HepaRG MTs require the presence of 0.5% DMSO in the culture media to exhibit bile canaliculi

comparable in surface area and structure to that observed in hLiMTs. Using confocal high content screening (HCS) and two bile transporter indicator dyes (CLF and CMFDA), it is possible to determine intrahepatic accumulation, reduced bile acid transport and corresponding dye accumulation by known cholestatic compounds. The BSEP inhibitors troglitazone, bosentan, ketoconazole and cyclosprin A showed either hepatic accumulation and/or concomitant bile canaliculi loss of CLF at a concentration comparable to BSEP vesicle assays (<20, 25-50, <25 and <5µM respectively) in both hLiMTs and HepaRG MTs. It was also shown that the predominantly MRP2 inhibitors benzbromarone and diclofenac (<40 and <50µM respectively) reduced the presence of CMFDA in bile canaliculi in both hLiMTs and HepaRG MTs, again comparable to vesicle assays. Both HepaRG and hLiMTs show similar responses, however a more comprehensive compound library would be required to establish the most predictive approach. This study shows how using a single organotypic liver MT per well and automated confocal HCS can be used as an early screen to predict drug cholestatic potential.

2011 Toxicity of Exifone in Primary Rat Hepatocytes and Human HepaRG Cells (clone 5f)

T. Brayman and D. Thompson. *Sigma-Aldrich, St. Louis, MO.*

Exifone is a hexahydroxybenzophenone that was developed to treat cognitive memory problems in the aged (nootropic). It was launched in April 1988 in France. However, it was removed from the market in May 1989 due to reports of several cases of hepatic injury, predominantly of a hepatocellular or mixed pattern, accompanied by marked cholestasis. A tentative estimate of the prevalence of hepatic reactions to exifone was 1 in 10,000-15,000 treated patients. Little is known about the mechanism of toxicity of exifone. In primary rat hepatocytes, exifone caused a concentration-dependent loss of cell viability, accompanied by a loss of intracellular glutathione. Concentrations of 300 µM resulted in loss of approximately 50% viability within 24 hr. Exifone was oxidized by both cytochrome p450 and tyrosinase to a reactive quinone metabolite ($\lambda max = 433$ nm) which reacted with glutathione. The rate of auto-oxidation in culture media was minimal over the 24 hr culture period. Exifone markedly increased the expression of the early response gene, Egr1, following 24 hr exposure. In addition, several genes related to oxidative stress were up-regulated, including Gpx6, Prdx1 and Nox4. Similar results were obtained in HepaRG cells - decrease in glutathione and loss of cell viability at 24 hr, accompanied by increased ROS generation. Taken together, these preliminary studies on exifone hepatotoxicity suggest that metabolic activation to a reactive metabolite and oxidative stress are early events in the response to exifone exposure.

2012 Mechanisms of Usnic Acid-Associated Hepatotoxicity in HepG2 Cells

L. Guo and S. Chen. *NCTR, Jefferson, AR.*

Crude extracts of usnic acid or pure usnic acid have been marketed in the United States to aid in weight loss; however, the use of usnic acid containing products has been reported to be associated with acute liver failure in humans. Using human HepG2 cells, we investigated and characterized the possible mechanisms, especially the contributions of apoptosis, ER stress, store-operated calcium entry, and autophagy in usnic acid's toxicity. Usnic acid caused apoptosis as demonstrated by an increased caspase-3/7 activity and an increased subdiploid nucleus formation. Usnic acid caused endoplasmic reticulum (ER) stress demonstrated by the increased expression of typical ER stress markers, including CHOP, ATF-4, p-eIF2a, and spliced XBP1. Usnic acid increased the cytosolic free Ca2+ concentration and increased the expression of calcium release-activated calcium channel protein 1 (ORAI1) and stromal interaction molecule 1 (STIM1), two key components of store-operated calcium. Caspase inhibitor and ER stress inhibitor attenuated usnic acid-induced cytotoxicity, indicating apoptosis and ER stress are the toxic mechanisms of usnic acid-associated toxicity. Moreover, usnic acid also induced autophagy as demonstrated by the conversion of LC3B-I to LC3B-II, degradation of P62, and an increased number of puncta. Inhibition of autophagy by treating cells with autophagy inhibitors (3-methyladenine or chloroquine) or by small interfering RNA against Atg7 aggravated usnic acid-induced apoptosis and decreased cell viability, indicating that autophagy plays a protective role against usnic acid-induced toxicity. Taken together, multiple pathways are involved in usnic acid-associated hepatotoxicity.

2013 Transcriptome Features of an HepaRG Cell Line Over-Expressing Human Flavin-Containing Monooxygenase-3 (FMO3)

S. Rudraiah, S. Piekos, W. Krueger, T. Rasmussen, X. Zhong and J. E. Manautou. *Pharmaceutical Sciences, University of Connecticut, Storrs, CT.*

Flavin-containing monooxygenase 3 (FMO3) is a transmembrane protein localized to the endoplasmic reticulum of many tissues and belongs to the second most important class of phase-1 drug-metabolizing enzymes. Recently we demonstrated liver Fmo3 gene induction and protein expression during acetaminophen (APAP) hepatotoxicity in mice and as well as APAP-induced cytotoxicity in human HepaRG cell line. In addition, results from FMO3 over expressing HepaRG cells (hFMO3-HepaRG) showed that changes in FMO3 gene expression not only alters HepaRG cell phenotype, as evidenced by an enhanced rate of cellular differentiation that is expression level-dependent, but also lowers susceptibility to APAP-induced cytotoxicity. However, cellular processes affected during FMO3-driven hepatocyte differentiation in HepaRG cells *in vitro* were not defined. Here, we performed RNA-sequencing and transcriptome analyses in hFMO3-HepaRG cells (high- and low-FMO3 expressing cell line clones) in comparison with empty vector controls. Strikingly, down-regulated genes, as opposed to genes that were up-regulated, correlated well with the differentiation phenotype in hFMO3-HepaRG cells. Gene ontology analysis revealed that down-regulated genes are associated with tissue remodeling processes including collagen catabolic and metabolic processes, extracellular matrix organization, extracellular structure organization, collagen fibril organization, negative regulation of cell fate specification and regulation of mesodermal cell fate specification. On the other hand, up-regulated genes are associated with sterol metabolic process, lipid biosynthetic process and steroid biosynthetic process. Taken together, these data reveal an unexpected potential novel role for FMO3 overexpression in tissue remodeling processes following APAP-induced liver injury. Characters: 2114/2300

2014 The Influence of System-Specific Factors on *In Vitro* Kinetics in 3D Flow-Based Liver Bioreactors

M. Yoon[2], J. M. Pedersen[2], E. L. LeCluyse[2], J. M. Macdonald[1], M. E. Andersen[2] and H. J. Clewell[2]. [1]*The University of North Carolina, Chapel Hill, NC and* [2]*Center for Human Health Assessment, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Three dimensional (3D) bioreactor systems have made possible long term hepatocyte cultures that maintain liver-like functions such as albumin production. However, declining xenobiotic metabolic enzyme activities over time and system specific restrictions such as non-specific binding are obstacles in arriving at reliable prediction of metabolism parameters with bioreactors. Here, two bioreactor systems, Quasi-Vivo® (QV) and RealBio® (RB), and three types of tubing (PharMed®, silicone, teflon) were used to identify major non-biological factors that affect the utility of 3D-flow based bioreactors for investigating metabolism. 7-ethoxycoumarin (7EC) and acetaminophen (APAP) was used as test compounds. The RB system equipped with a 3D scaffold for cell attachment and PharMed® tubing for fluid circulation showed large variability in quantitative measurements likely due to cell attachment variability and significant non-specific binding to the tubing. Alginate hydrogel-supported 3D culture was introduced to the QV system to increase cell to media ratio. This modification improved system reproducibility in quantitative measurements. In addition, teflon tubing, showed the lowest non-specific binding for 7EC and APAP among the three tested tubing. Silicone had the highest compound adherence with low reversibility. PharMed® tubing had high but reversible binding of the test compounds. Our results underline the importance of accounting for system specific factors when designing and using bioreactors for metabolism studies. Although it is not possible to eliminate these factors, their contribution to compound kinetics in a bioreactor can be accounted for by biokinetic modeling to ensure proper interpretation of the results for predicting *in vivo* metabolism parameters. (Funded by American Chemistry Council's Long-Range Research Initiative)

2015 Exploration of Drug-Induced Mitochondrial Toxicity Mechanisms on Hepatic Mitochondria and Cultured Cells

R. Note[1], A. Crudu[1], A. Riu[1], M. Porceddu[2], C. Pertuiset[2], N. Buron[2], A. Borgne-Sanchez[2] and S. Teissier[1]. [1]*L'Oréal, Aulnay-sous-Bois, France and* [2]*Mitologics S.A.S., Paris, France.* Sponsor: E. Dufour.

Drug-induced hepatotoxicity is mediated by different mechanisms. However, mitochondrial dysfunction is considered as a key mechanism in this toxicological outcome. The development of *in vitro* screening techniques could represent a major breakthrough for a rapid selection of safer compounds. Hence, we studied the toxicity mechanism of six reference drugs (Acetaminophen, Amiodarone, Stavudine, Thapsigargin, Troglitazone and Valproic acid) on MiToxView® screening platform by using both isolated HepaRG mitochondria and differentiated HepaRG cultured cells. On isolated mitochondria, swelling, transmembrane potential loss, inhibition of oxygen consumption driven by activation of complex I, complex II or fatty acids β-oxidation (FAO) and activities of respiratory chain complexes were investigated to determine potential acute and direct mitochondrial toxicity of the tested drugs. Potential long-term and indirect effects were investigated on differentiated HepaRG cells after 2, 7 and 12 days of treatment by measuring cell viability, mitochondrial transmembrane potential, ADP/ATP ratio, global respiration, mitochondrial ROS production and mtDNA depletion. These multiparametric assays on hepatic mitochondria and cultured cells highlighted different mechanisms of mitochondrial toxicity of the 6 reference drugs: direct and acute toxicity (Amiodarone, Thapsigargin, Troglitazone), direct and long term toxicity of the parent drug (Stavudine) or its metabolites (Acetaminophen, Valproate), occurring before cytotoxicity. We have demonstrated that the MiToxView® could be used to fully understand the mechanism of mitochondrial alterations. Given the central role of mitochondria in cell homeostasis, the reliance on such a screening platform could be helpful for the early assessment of general toxicity associated with new cosmetic ingredients.

2016 *In Vitro* Models for the Prediction of Idiosyncratic Drug Induced Liver Injury

A. Granitzny[1], J. Knebel[1], C. Dasenbrock[1], P. Steinberg[2] and T. Hansen[1]. [1]*Fraunhofer ITEM, Hannover, Germany and* [2]*University of Veterinary Medicine Hannover, Hannover, Germany.*

An important subset of drug-induced liver injury (DILI) is the idiosyncratic form (iDILI), which is rare but particularly severe and accounts for about 13% of all liver failure cases. Since iDILI cannot be predicted by non-clinical animal studies, validated human *in vitro* models are urgently needed. Although the mechanisms of iDILI are not fully understood, several studies point to an involvement of immune reactions and inflammatory stress. In order to evaluate this hypothesis, which suggests a critical role of immune cells and its secreted factors, we established an *in vitro* co-culture system combining human monocytic (THP-1) with human hepatoma (HepG2) cells. With this model we aimed to identify whether the introduction of inflammatory immune cells, like monocytes, in the presence or absence of pro-inflammatory factors (LPS, TNFα) increases the sensitivity of liver cells towards iDILI compounds. Therefore, we chose three reference substance pairs (Troglitazone - Rosiglitazone; Trovafloxacin - Levofloxacin; Diclofenac - Acetylsalicylic Acid), each of them being composed of a compound that is known to induce iDILI and a partner compound of the same substance class that does not induce iDILI. The compounds were tested alone or in combination with LPS or TNFα in the HepG2 cell monoculture or in the HepG2-THP-1 co-culture. By using the WST-assay, we observed that each iDILI compound showed a significant increase in cytotoxicity at least in one exposure combination, while this effect was not observed with the corresponding non-DILI partner compound. We conclude that a combination of different cell culture systems and the co-exposure with pro-inflammatory factors is needed for a valid differentiation between non-DILI and iDILI compounds. This test battery could provide a useful tool for the prediction of inflammation-associated idiosyncratic drug-induced hepatotoxicity.

2017 Lack of Adequate Classification of Hepatotoxicants Hinders Development of Predictive *In Vitro* DILI Screening Assays

D. Thompson. *Sigma-Aldrich, St. Louis, MO.*

Early detection of drug-induced liver injury (DILI) remains a major concern in the drug development process and considerable effort has been devoted over the past few years toward identifying predictive *in vitro* screening assays to address this issue. A significant challenge for such studies, however, is the choice of compounds for use in validation of new assays. Analysis of several recently published assays for hepatotoxicity revealed highly divergent lists of compounds considered as "positive" and/or "negative" controls. Some studies used lists of drugs with black box or other warnings for hepatotoxicity as positives, while others included drugs culled from literature reports of hepatotoxicity or various public databases. A common caveat with these lists, however, is that there is sparse information about the incidence of hepatotoxicity with most of the drugs. For example, many of these lists mix intrinsic with idiosyncratic toxicants. More specifically, a number of very low incidence compounds are being included as positives for the assay. One of the unfortunate results of this type of approach is that the potential usefulness of a novel assay cannot be properly evaluated and that effects of very low incidence compounds may be misinterpreted as predictive signals of toxicity. This particularly impacts sensitivity and specificity tables which are commonly used to describe the predictivity of the assay. A more realistic approach for assessing the predictive value of a new assay would be to separate model compound sets based on their incidence of injury. A collaborative effort is required to obtain and share incidence data and to determine cutoffs for inclusion of compounds as positive or negative controls. Overall, the predictivity of current *in vitro* DILI assays may be greatly improved if a common set of hepatotoxic compounds, based on incidence, were used as positive controls.

2018 Application of an *In Vitro* High Content Imaging Assay for Quantitative Assessment of Rat, Mouse, and Human Primary Hepatocyte Proliferation

V. Soldatow[2], R. Peffer[1], D. Cowie[1], K. Lichti-Kaiser[1], R. Clewell[2], M. Andersen[2], E. LeCluyse[2] and C. Deisenroth[2]. [1]*Syngenta Crop Protection, LLC, Greensboro, NC and* [2]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Rodent liver tumors promoted by nuclear receptor activation (CAR, PXR, PPARα, AhR) are known to be mediated by key events that include hepatocellular proliferation and suppression of apoptosis. Understanding the human relevance of these modes of action using an *in vitro* model has been hampered by challenges with reproducing proliferative responses in cultured primary hepatocytes. Here, an *in vitro* high content imaging based assay was developed for quantitative assessment of nascent DNA synthesis in primary hepatocyte cultures from mouse, rat, and human. Detection of DNA synthesis was performed using direct DNA labeling with the nucleoside analog 5-ethynyl-2′-deoxyuridine (EdU). The assay was multiplexed to enable direct quantitation of DNA synthesis, cytotoxicity, and cell count endpoints. An optimized defined medium cocktail was developed to sensitize hepatocytes to cell cycle progression. The baseline EdU response to defined medium was greatest for mouse, followed by rat, and then human, revealing the differential sensitivities of each species to mitogenic stimulus *in vitro*. Hepatocytes from all three species demonstrate CAR activation via Cyp2B induction by species-dependent CAR agonists TCPOBOP, CITCO, or Phenobarbital. When evaluated for a proliferation phenotype, TCPOBOP and CITCO exhibited significant dose-dependent increases in frequency of EdU labeling in mouse and rat hepatocytes that was not observed in hepatocytes from three human donors. The results demonstrate a multiplexed *in vitro* phenotypic assay for hepatocyte DNA synthesis with species differences in proliferative response that are consistent with prior published work indicating rodent liver tumors generated via CAR activation are not relevant to humans.

2019 The Non-Narcotic Antitussive Benzonatate is not Carcinogenic in Rodent Assays

S. Teo[3], M. McKeon[1] and S. Lee[2]. [1]*BioReliance, Rockville, MD;* [2]*Covance, Madison, WI and* [3]*Pfizer, Madison, NJ.*

Benzonatate (TESSALON®) is a non-narcotic, peripherally acting, oral prescription antitussive for the symptomatic relief of cough. It is an ester type anesthetic structurally related to procaine. Benzonatate (B) is thought to inhibit cough by anesthetizing pulmonary stretch receptors which mediate the cough reflex. Benzonatate is rapidly metabolized by body esterases to the major metabolite 4-(butylamino)benzoic acid (BBA). Carcinogenicity assays were performed using hemizygous Tg.rasH2 mice and Wistar (Han) rats. In the 6-month Tg.rasH2 mouse study, male mice (25/group) were orally dosed with vehicle (deionized water), 10, 30, 75 or 100 mg/kg/day, and female with vehicle, 5, 15 or 50 mg/kg/day. Doses were based on 28-day dose ranging study in CByB6F1 (Tg.rasH2 non-transgenic littermate) mice. Exposure was confirmed by toxicokinetic plasma sampling at Week 14 with undetectable levels of B and significant amounts of BBA. The 50 and 100 mg/kg doses for female and male mice have free plasma BBA AUCs that were 11- and 19-times respectively higher than that from a maximum daily human dose of 600 mg. There were no dose limiting toxicities in the 6-month study. Positive control mice (10/sex) were treated by intraperitoneal injection (Days

1, 3, 5) with 1000 mg/kg urethane. Statistically significant increases in the incidence of splenic hemangiosarcomas and combined incidence of all hemangiosarcomas were observed in the 30 mg/kg/day males. This was not considered BBA-related due to a similar incidence in the historical control database and the lack of dose dependent response or trend. No other significant increases in malignant and non-malignant tumors or toxicity were detected for the B-treated mice. In the 2-year rat study, male Wistar (Han) rats were orally dosed with vehicle (water), 10, 30 or 90 mg/kg/day and female rats with 5, 15 or 50 mg/kg/day. Doses were based on a 3-month dose ranging study. Exposure was confirmed by toxicokinetic plasma sampling at Week 26 with undetectable levels of B and significant amounts of BBA. The 50 and 90 mg/kg/day doses for female and male rats have free plasma BBA AUCs that were 30- and 22-times higher respectively than that from a maximum daily human dose of 600 mg. No BBA-related increases in malignant and non-malignant tumors or toxicity were detected. In summary, rodent assays determined that the major B metabolite BBA was not carcinogenic at exposures that were at least 11 times higher than that from a maximum daily human dose.

2020 Peroxisome Proliferator-activated Receptor-β/δ (PPARβ/δ)-dependent Inhibition of UVB-induced Skin Carcinogenesis: Targeting p53 Mutant Cells

D. H. Ralph[1], K. Pramanik[1], F. J. Gonzalez[2] and J. M. Peters[1]. [1]*Dept. of Veterinary and Biomedical Sciences and Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA and* [2]*Laboratory of Metabolism, National Cancer Institute, Bethesda, MD.*

Non-melanoma skin cancer is the most common type of cancer in the U.S. with 5 million people treated for it each year. Approximately 90% of non-melanoma skin cancers are caused by UV-induced sun damage. Past studies have shown that ligand activation of peroxisome proliferator-activated receptor β/δ (PPARβ/δ) inhibits chemically-induced skin cancer. The hypothesis that ligand activation of PPARβ/δ inhibits UVB-induced skin cancer was examined in this study. Female SKH-1 mice (wild-type or Pparβ/δ-null) were UVB irradiated (180 mJ/cm2) 3 times/week followed by topical application with vehicle control (acetone), the highly specific PPARβ/δ ligand (GW0742: 1.0, 2.5, or 5.0 uM), or the natural PPARβ/δ ligand (oleic acid: 25, 50, or 100 uM). The onset of tumor formation and tumor multiplicity were significantly decreased in response to ligand activation of PPARβ/δ as compared to control, and these effects were not found in Pparβ/δ-null mice. To begin to determine the mechanisms for the chemopreventive effects of ligand activation of PPARβ/δ, female SKH-1 mice (wild-type or Pparβ/δ-null) were UVB irradiated (30 mJ/cm2) 2 times/week directly followed by topical application with vehicle control (acetone), the synthetic or natural PPARβ/δ ligands as described above. After 20 weeks, epidermal sheets were isolated and immunohistochemical analysis was performed to detect cellular patches (>8 adjacent cells/patch) of mutant p53. UVB irradiation caused an increase in mutant p53 patches as compared to controls, and ligand activation of PPARβ/δ significantly decreased the number of p53 mutant patches compared to control. Similarly, UVB irradiation caused an increase in pyrimidine (6-4) pyrimidone photodimers and this effect was also reduced by ligand activation of PPARβ/δ with both the synthetic and natural ligands. Results from these studies provide strong evidence that ligand activation of PPARβ/δ prevents UVB-induced skin cancer by targeting p53 mutant cells and inhibiting UVB-induced DNA damage. (Supported by CA124533, CA140369).

2021 Ligand Activation of PPARβ/δ Inhibits EMT in a Human Colon Cancer Cell Line

K. Consevage[2], K. Praminick[2], F. Gonzalez[1] and J. Peters[2]. [1]*National Cancer Institute, Bethesda, MD and* [2]*Penn State University, State College, PA.*

Colorectal cancer patients with relatively higher expression of peroxisome proliferator-activated receptor β/δ (PPARβ/δ) in their primary tumors as compared to patients with relatively lower PPARβ/δ expression exhibit lower propensity of metastasis and significantly increased survival. One of the mechanisms underlying cancer metastasis is the process of epithelial-to-mesenchymal transition (EMT). During EMT, cells lose their epithelial phenotype while gaining characteristics of mesenchymal cells. This study tested the hypothesis that ligand activation of PPARβ/δ would inhibit EMT using a human colon cancer cell line (HCT116). In this study, tumor necrosis factor α (TNFα-20 ng/mL) was used to induce EMT in HCT116 cells. The effect of ligand activation of PPARβ/δ on EMT in HCT116 cells was determined by culturing cells with and without the highly specific PPARβ/δ agonist, GW0742 (0.1 μM and 1.0 μM). HCT116 cells cultured in TNFα developed a mesenchymal-like morphology, beginning at 12 hours post-TNFα treatment and becoming more evident at 36 hours after TNFα treatment. Consistent with this phenotype, TNFα also caused a decrease in E-CADHERIN mRNA and protein expression and an increase in N-CADHERIN protein expression in HCT116 cells compared to controls. Ligand activation of PPARβ/δ with GW0742 caused an increase in angiopoietin-like 4 (ANGPTL4) mRNA levels, a well-characterized PPARβ/δ target gene. Co-culturing HCT116 cells with the PPARβ/δ agonist, GW0742 and TNFα caused a decrease in TNFα-induced N-CADHERIN expression and an increase in E-CADHERIN protein expression compared to TNFα controls. However, there were no grossly apparent differences in cell morphology. Ligand activation of PPARβ/δ with GW0742 did not affect basal E-CADHERIN expression, but caused a decrease in basal N-CADHERIN expression in HCT116 cells compared to controls. Collectively, results from this study provide evidence that ligand activation of PPARβ/δ attenuates TNFα-induced EMT in human colon cancer cells. (Supported by CA124533, CA140369).

2022 A Highly Sensitive Bioluminescence-Based Assay for Quantifying Cancer Cell Load in Target Organs: Implications for Studying Anticancer Drugs, Including Natural Redox-Active Quinones

H. Zhu[1], M. E. Kauffman[1], J. Z. Li[2], S. Sarkar[1], M. A. Trush[3], Z. Jia[4] and Y. R. Li[1]. [1]*Campbell University School of Osteopathic Medicine, Buies Creek, NC;* [2]*College of Arts and Science, University of Virginia, Charlottesville, VA;* [3]*Johns Hopkins University Bloomberg School of Public, Baltimore, MD and* [4]*Biology, University of North Carolina at Greensboro, Greensboro, NC.*

Animal models are essential for developing effective anticancer drugs for humans. Examination of lung surface foci of B16-F10 melanoma cells is a widely used animal model for studying cancer metastasis and drug intervention. However, this model suffers several drawbacks, including the non-quantitative feature and lack of information on cancer cell load inside the lung tissue. Here we report the development of a highly sensitive, bioluminescence-based assay for quantifying melanoma cell load in the mouse lungs following intravenous injection of luciferase-expressing B16-F10 melanoma cells. This assay could readily detect as few as 1-10 cells in the samples and enable quantification of the cancer cell load before formation of surface foci in the mouse lungs following metastasis of the intravenously inoculated B16-F10 melanoma cells. This innovative quantitative assay has important implications for studying anticancer drugs, including naturally occurring redox-active quinones.

2023 A Nutrient Mixture Modulates Ovarian ES-2 Cancer Progression by Inhibiting Xenograft Tumor Growth and Cellular MMP Secretion, Migration and Invasion

M. W. Roomi, N. W. Roomi, A. Niedzwiecki and M. Rath. *Dr. Rath Research Institute, Santa Clara, CA.*

Epithelial ovarian carcinoma, which occurs mainly in post-menopausal women, is the leading cause of death from gynecological malignancy and the fifth most common cancer in the U.S. Since ovarian cancer often remains clinically silent, the majority of patients with ovarian carcinoma have advanced intraperitoneal metastatic disease at diagnosis, resulting in a poor prognosis. Long-term survival of patients with ovarian cancer remains poor, due to metastasis and recurrence. We investigated the effect of a nutrient mixture (EPQ) containing ascorbic acid, lysine, proline, quercetin and green tea extract *in vivo* and *in vitro* on human ovarian cancer ES-2 cell line. *In vivo*, athymic female nude mice (n=12) were inoculated with 3x106 ES-2 cells subcutaneously and randomly divided into two groups: group A was fed a regular diet and group B a regular diet supplemented with 0.5% EPQ. Four weeks later, the mice were sacrificed and their tumors were excised, weighed and processed for histology. Dimensions (length and width) of tumors were measured using a digital caliper, and the tumor burden was calculated using the following formula: 0.5 x length x width. We also tested the effect of EPQ *in vitro* on ES-2 cells, measuring cell proliferation by MTT assay, MMP secretion by zymography, invasion through Matrigel, migration by scratch test and morphology by H&E staining. EPQ inhibited tumor weight and burden of ES-2 tumors by 59.2% ($p < 0.0001$) and 59.7% ($p<0.0001$), respectively. *In vitro*, EPQ exhibited 35% ($p<0.0001$) toxicity over the control at 1000 μg/ml concentration. Zymography demonstrated only MMP-2 with and without PMA, which was inhibited by EPQ in a dose dependent fashion, with near total inhibition at 1000 μg/ml. Migration by scratch test and Invasion through Matrigel were inhibited in a dose dependent manner with total block of invasion and migration at 500 μg/ml. These results suggest that EPQ has therapeutic potential in treatment of ovarian cancer.

2024 Peroxisome Proliferator-Activated Receptor-β/δ (pparβ/δ) Inhibition of Human Neuroblastoma Cell Tumorigenesis Is Associated with P53-Dependent Cell Differentiation

P.-L. Yao[2], L. Chen[2], J. Singh[2], T. P. Dobrzański[2], M. E. Campbell[2], J. A. Krevitz[2], B. Zhu[2], F. J. Gonzalez[1], US Singh[3] and J. M. Peters[2]. *[1]National Cancer Institute, Bethesda, MD; [2]Pennsylvania State University, University Park, PA and [3]University of South Carolina, Columbia, SC.*

Neuroblastoma is a common childhood tumor developed from immature nerve cells. The differentiation stage of neuroblastoma correlates with aggressiveness of this disease. Peroxisome proliferator-activated receptors β/δ, (PPARβ/δ) regulates a variety of biological processes including cell differentiation. Three human neuroblastoma cell lines, NGP, SK-N-BE(2) and IMR-32, representing high, medium and low sensitivity to retinoic acid, were used to constitutively over-express PPARβ/δ. These cell lines were used to examine the role of this receptor in human cancer models. PPARβ/δ-dependent inhibition of cell proliferation was observed in NGP cells, but not in SK-N-BE(2) and IMR-32 cells. All-trans retinoic acid (atRA) decreased expression of SOX2, a stem cell marker, in NGP and SK-N-BE(2) cells exhibiting inactive and mutant p53, respectively. However, atRA did not suppress SOX2 expression in IMR-32 cells containing wild-type p53. Over-expression and/or ligand activation of PPARβ/δ reduced average tumor volume and tumor weight of ectopic xenografts from NGP, SK-N-BE(2) or IMR-32 cells compared to controls. Interestingly, PPARβ/δ-suppressed SOX2 expression in IMR-32 cells *in vitro* and *in vivo* and was associated with the increased p53 expression, but this inhibitory effect was limited in NGP and SK-N-BE(2) cells. Additionally, attenuation of tumor growth in NGP cells was associated with a PPARβ/δ-dependent decrease in PCNA expression, and increased necrosis and local neutrophil infiltration. Combined, these novel observations demonstrate the inhibitory effect of activating or over-expressing PPARβ/δ on tumorigenesis by inducing cell differentiation through a p53-dependent SOX2 signaling, implicating an alternative therapeutic application to retinoic acid-resistant neuroblastoma patients. (Supported by CA124533, CA140369).

2025 Soy Protein Opposes Ethanol-Induced β-catenin Signaling and Tumor Promotion in a Mouse Model of Hepatic Cancer

C. Pulliam[3], K. Mercer[1], L. Hennings[1], K. Lai[1], K. Pedersen[3] and M. Ronis[3]. *[1]Arkansas Children's Nutrition Center, University of Arkansas for Medical Sciences, Little Rock, AR; [2]Pathology, University of Arkansas for Medical Sciences, Little Rock, AR and [3]Pharmacology & Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, LA.*

Alcohol is a risk factor for hepatic cancer, which is often associated with modified β-catenin signaling. As soy diets may be cancer preventative, we hypothesized that soy protein counteracts ethanol-induced hepatic β-catenin signaling. After tumor initiation with diethyl nitrosamine, mice were for 16 weeks fed standard chow or Lieber-DeCarli (LD) diets with +/- 28% of carbohydrate calories replaced with ethanol. The protein was either casein or soy protein isolate. In ethanol-fed mice, soy reduced adenoma incidence relative to casein ($P<0.05$), ameliorated necroinflammatory injury and fibrosis, inhibited hepatocyte proliferation ($P<0.05$), and exhibited reduced Wnt/β-catenin signaling with decreased GSK3β phosphorylation, reduced nuclear β-catenin content, and decreased expression of Cyclin D1 mRNA ($P<0.05$). Liver RNA was further analyzed with a PCR array for the Wnt/β-catenin pathway. The pattern is complex with contrasts between diets involving relatively small changes in mRNA of both stimulating and inhibiting signaling factors. Yet, the expression of three genes inhibiting signaling, Kremen1, Fbxw4 and Fbxw11, were induced by soy in ethanol-fed mice ($p<0.05$). For two β-catenin target genes, c-Jun and Cyclin D1, there was significant interaction between the protein source and ethanol, with soy only decreasing expression in the presence of ethanol. Conclusion: Soy protein has primarily post-transcriptional inhibitory effects on Wnt/β-catenin signaling, which may ultimately lead to reduced Cyclin D1 expression and proliferation. Support: R21 CA 169389 (M.R.).

2026 Role of the Long Non-Coding RNA MALAT-1 in Pancreatic Cancer

Y. Cheng[4], P. Imanirad[4], I. Jutooru[1], A. Hoffmann[4], B. Morpurgo[4] and A. E. Golovko[4]. *[1]Covance, Inc, Madison, WI; [2]TIGM, Texas A&M University, College Station, TX; [3]VTPB, Texas A&M University, College Station, TX and [4]VTPP, Texas A&M University, College Station, TX.*

Long non-coding RNAs (Lnc RNAs) contain > 200 nucleotides and based on limited studies there is evidence that they regulate multiple functions and contribute to cellular homeostasis and diseases including cancer. The lncRNA Metastasis-Associated-in-Lung-Adenocarcinoma-Transcript-1 (MALAT-1) is overexpressed in pancreatic and other cancer cell lines and tumors and is a negative prognostic factor for multiple cancers. MALAT-1 function was investigated by RNAi in Panc-1 and Miapaca2 pancreatic cancer cell lines and knockdown of MALAT-1 (si-MALAT-1) decreased the cell viability, migration/invasion, induced G2/M cell cycle arrest and apoptosis, which suggest that MALAT-1 plays a role in pancreatic cancer cell survival. Panc1 cells were transfected with siCtrl (control siRNA), siMALAT-1 and also oligonucleotide targeting MLL-1 and EZH2 which are important components of histone modification complexes. Functional and pathway analysis for differential expressed genes after knocking down of MALAT-1, MLL-1 or EZH2 were analyzed by Ingenuity software and showed that MALAT-1 is involved in multiple pathways including those associated with cell death, proliferation and migration/invasion, and the interferon signaling was most significant pathway from the canonical pathway analysis. Venn diagram analysis showed that 107 common genes were co-upregulated by siMALAT-1 or siEZH2 treatment, and 75 genes were co-downregulated by siMALAT-1 or siMLL1 treatment. Some representative coregulated genes related to cell migration and invasion were further investigated by real time PCR, confirming that expression of both SMAD3 and EZR were decreased after knockdown of MALAT-1 or MLL1, and sprouty2 (SPRY2) and NDRG1 expression were increased after knockdown of MALAT-1 or EZH2. We have also generated heterozygous floxed p53/KrasGD12 mice (p53L/+: LSL-KrasGD12L/+:p48Cre+/-) which spontaneously develop pancreatic tumors, and these have been crossed with MALAT-/- mice. A comparison of the lifespan of these mice expressing wild-type or deleted MALAT-1 (homo plus heterozygote mice) showed that with the loss of one or both MALAT-1 alleles, there was an increase in lifespan demonstrating an *in vivo* pro-oncogenic role of MALAT-1 in a mouse model of pancreatic cancer.

2027 Protective Effect of Quercetin Against Benzo[a] pyrene-Induced DNA Damage in Sprague-Dawley Rats

H. S. Kim and K. S. Kim. *School of Pharmacy, Sungkyunkwan University, Suwon, Korea, Republic of.* Sponsor: H. S. Kim.

Benzo[a]pyrene (BaP), a five-ring polycyclic aromatic hydrocarbon, is a well-known as environmental pollutant. It has been reported that dietary exposure to benzo(a)pyrene (BaP) can be induced the gastrointestinal carcinogenesis. Thus, it is necessary to evaluate the effect of phytochemicals on gastric cancer initiation and progression. In this study, we investigated the preventive effects of quercetin on BaP-induced DNA damage in Sprague-Dawley rats. The flavonoid quercetin is naturally found in fruits and vegetables which have physiological properties such as antioxidant, anti-aging, and anti-inflammatory effects. For the first group, BaP (20 mg/kg body weight) was administered to rats in corn oil via oral gavage over a 30 days period. For the second group, quercetin (2.5, 7.5, or 15 mg/kg) was coadministered with BaP for 30 days. All rats were anesthetized and major organs, including liver, kidney, stomach, testis, and epididymis were removed and examined 24 h after the last treatment. Blood urea nitrogen (BUN) and aspartate aminortansferase (AST) levels were significantly increased in the BaP-treated group when compared to control group. Expression levels of CYP1A1 and CYP1B1 were significantly increased in the liver of rats treated with BaP. However, cotreatment with quercetin markedly decreased CYP1A1 and plasma concentration of BPDE compared with BaP alone. Furthermore, cotreatment with quercetin significantly decreased the BPDE-I-DNA adduct formation in liver and stomach. Overall, our observations reveal that quercetin inhibits gastric tumorigenesis when given together with BaP and holds promise as a chemopreventive agent.

2028 Epigenetic Downregulation of Mitochondrial Oxidative Phosphorylation-Related TMEM70 From an Early Stage of Hepatocarcinogenesis in Rats

S. Mizukami[1,2], A. Yafune[1,2], M. Kimura[1,2], Y. Hasegawa-Baba[1], T. Yoshida[1] and M. Shibutani[1]. [1]*Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo, Japan and* [2]*Pathogenic Veterinary Science, United Graduate School of Veterinary Science, Gifu University, Gifu, Japan.* Sponsor: M. Ema.

Genomic methylation status can be inherited by daughter cells, and alteration of DNA methylation in the genome is found in almost all types of cancers and can lead to changes in gene expression to acquire further growth advantages for cancer development. To obtain irreversible molecular markers from the early stage of hepatocarcinogenesis, CpG island microarray analysis was performed in the liver of rats treated with a hepatocarcinogen, thioacetamide (TAA), for 28 days. Then, candidate molecules obtained from hypermethylated gene profile were immunohistochemically examined in terms of distribution in association with preneoplastic and neoplastic lesions produced by tumor promotion with hepatocarcinogens/promoters in a two-stage heptocarcinogenesis model. Among 48 genes hypermethylated, Tmem70, encoding mitochondrial membrane protein playing a role in biogenesis of ATP synthase, was confirmed to be hypermethylated by methylation-specific PCR and pyrosequencing analyses accompanying downregulation of mRNA level by real-time RT-PCR. Immunohistochemically, TMEM70 showed cytoplasmic immunoreactivity in non-neoplastic liver cells, but immunoreactivity of TMEM70 was decreased in preneoplastic liver cell foci positive for glutathione S-transferase placental form (GST-P), a preneoplastic liver cell marker, produced by tumor promotion. The incidence of TMEM70-/GST-P+ foci, as well as ATP synthase complex V beta subunit (ATPB)-/GST-P+ foci, was increased with tumor promotion by all hepatocarcinogens/promoters. In contrast, glycolysis-related molecules, glucose transporter 1 (GLUT1) and glucose-6-phosphate dehydrogenase (G6PD), showed co-expression with GST-P and the incidences of GLUT1+/GST-P+ and G6PD+/GST-P+ foci were increased by tumor promotion. Downregulation of TMEM70 and ATPB and upregulation of G6PD were also obtained in GST-P+ hepatocellular tumors induced after long-term promotion treatment. These results suggest that Tmem70 may play a role as a tumor suppressor gene showing epigenetic downregulation from an early stage of hepatocarcinogenesis to contribute to metabolic shift from oxidative phosphorylation to glycolysis.

2029 Enhanced Phosphorylation of H3ser10 and H3ser28 Involves in Chemical Carcinogen-Induced Cell Transformation

X. Zhu, Z. Zhang, L. Chen and W. Chen. *Toxicology, Sun Yat-sen University, Guangzhou, China.* Sponsor: W. Chen.

Epigenetic alterations including histone modification have been implicated in carcinogenesis and cell transformation. Despite several post-translational modifications on histone H3 have been reported to be activated by stress to trigger multiple tumor-related signaling pathways, the role of histone H3 phosphorylation playing in chemical carcinogenesis remain not fully understood. Here we showed that histone H3 phosphorylation at Ser10 (p-H3Ser10) and Ser28 (p-H3Ser28) were up-regulated in aflatoxin B1-induced transformed L02 cells (L02RT-AFB1), benzo(a)pyrene-transformed HBE cells (HBERT-BaP), and coke oven emissions-transformed HBE cells (HBERT-COE). In addition, they were highly expressed in 60.0% (6/10), 55.6% (5/9) of hepatocellular carcinoma (HCC), and 88.9% (8/9), 80.0% (4/5) of primary lung cancer, respectively. Using a specific phosphomimetic site mutation approach, we conducted functional studies showing that these mutated cell lines had no difference in proliferation and chromosome number, but affected denser chromosomes, less micronuclei and shorter comet tail moment formed after DNA damage. Moreover, these mutated cell lines showed prolonged transformation duration, demonstrated by less colonies formed in soft agar and less tumors grew in nude mice after AFB1-induced cell transformation. Importantly, down-regulation of p-H3S10 and p-H3S28 suppressed the ability for growing in soft agar and forming tumors in immunodeficient mice. Furthermore, we found that protein phosphatase 2A (PP2A) was responsible for dephosphorylation of H3Ser10 in AFB1-transformed cells. Particularly, we found that the substitution of H3S10 to alanine abolished the enrichment of promoters of tumor-related DNA damage repair (DDR) genes including mutL homolog 1 (MLH1) and poly ADP-ribose polymerase 1 (PARP1) at site of p-H3S10 modification. Taken together, we suggest that aberrant phosphorylation of H3S10 is involved in chemical-carcinogenesis by regulating the transcription of DDR genes.

2030 A Multiple Database Approach for Identifying Chemicals That Affect Cancer Pathways

S. Iyer[1], N. Pham[3], M. Marty[3], M. Sandy[1], G. Solomon[3] and L. Zeise[1]. [1]*Office of Environmental Health Hazard Assessment, CalEPA, Oakland, CA;* [2]*Office of Environmental Health Hazard Assessment, CalEPA, Sacramento, CA and* [3]*Office of the Secretary, CalEPA, Sacramento, CA.*

Several hundred chemicals have been identified as carcinogens by scientific entities, yet many thousands of chemicals have never been evaluated for carcinogenic potential. Chemical and toxicogenomic databases may be useful resources for quickly identifying and prioritizing bioactive environmental chemicals. Using chemical activity data from the US EPA ToxCast™ program's BioSeek platform, the Comparative Toxicogenomics Database™ (CTD), and expert judgement, we developed a novel screening approach to identify and prioritize chemicals with possible carcinogenicity concern. We identified 31 candidate ToxCast assay endpoints potentially related to cancer pathways. Of these assay endpoints, 21 have at least 13 curated associations with cancer in the CTD, as of its update on August 17, 2015, and 10 that are related to cell proliferation were selected based on expert judgement. Of the 1059 chemicals tested in ToxCast, 566 were active in at least one of the selected cancer-related assays. The 566 chemicals were ranked in activity, which included both assay quantity and potency. The ranking was based on a sum of chemical-assay AC50 and inactive values. Inactive chemical-assay pairs are assigned the value "1000" by US EPA. The 50% of chemicals most active were primarily pesticides and pharmaceuticals (n=221). The remaining 62 chemicals included chemical intermediates, surfactants, antioxidants, flame retardants, and plasticizers. The structures of the 5% of chemicals most active in our screen (n=29) were analyzed using the ChemoTyper application, and were found to contain 155 (out of 729 total) chemotypes. Three of these chemicals are recognized as carcinogens and listed under California's Proposition 65 as causing cancer: triphenyltin hydroxide, captafol, and kepone. This screening approach could be applied to an expanded set of cancer-related assay endpoints in ToxCast to identify and prioritize chemicals that may be of carcinogenic concern.

2031 Mode of Action of Ethyl Tertiary-butyl Ether in the Rat Liver: Induction of Oxidative Stress, Cell Proliferation and Cell Cycle Arrest via Activation of CAR, PXR and PPARs

A. Kakehashi[5], M. Gi[5], H. Wanibuchi[5], A. Hagiwara[1], N. Imai[1], K. Nagano[4], F. Nishimaki[3] and S. Fukushima[2]. [1]*DIMS Institute of Medical Science, Ichinomiya, Japan;* [2]*Japan Bioassay Research Center, Japan Industrial Safety & Health Association, Kanagawa, Japan;* [3]*Japan Petroleum Energy Center, Tokyo, Japan;* [4]*Nagano Toxicologic-Pathology Consulting, Kanagawa, Japan and* [5]*Dept. Molecular Pathology, Osaka City University Graduate School of Medicine, Osaka, Japan.*

To determine mode of action (MOA) for the non-genotoxic chemical, ethyl tertiary-butyl ether (ETBE) tumorigenicity in the rat liver, formation of oxidative stress, DNA repair, alteration to protein expression, cell ultrastructure, cell proliferation and apoptosis were investigated after ETBE administration to male F344 rats at doses of 300 and 2000 mg/kg/day by gavage and compared with effects induced by non-genotoxic carcinogen phenobarbital (PB, 500 ppm in diet). In the rat livers with centrilobular hypertrophy, significant increases of P450 total content and hydroxyl radical level after 7 days of ETBE administration, and elevation of 8-OHdG formation and peroxisome proliferation in hepatocytes at day 14, were accompanied by accumulations of CYP2B1/2B2, CYP3A1/3A2, CYP2C6, CYP2E1 and CYP1A1, downregulation of DNA oxoguanine glycosylase 1, and preceded by induction of cell proliferation at day 3. Furthermore, regenerative cell proliferation was observed as a result of ETBE treatment at day 28, after the induction of cell cycle arrest and apoptosis by day 14. From the results of proteome and IPA analyses, ETBE activated CAR, PXR and PPARs receptors. Thus, present results demonstrate that MOA of ETBE hepatotumorigenicity in rats is ascribed to overexpression of CYP2B/3A family, induction of oxidative stress, 8-OHdG formation, short-term elevation of cell proliferation, followed by subsequent cell cycle arrest, apoptosis and regenerative cell proliferation, predominantly by activation of CAR and PXR nuclear receptors by a mechanism similar to that of PB, and differentially by activation of PPARs.

2032 Design of a Rofecoxib-Combretastatin Hybrid Drug That Exerts Potent and Improved Antimicrotubule and Anti-Angiogenesis Properties Retaining Cox-2 Inhibition

H. R. Madala, S. R. Punganuru, M. Constantinos and S. Kalkunte. *Texas Tech University Health Sciences Center, Amarillo, TX; Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Amarillo, TX.*

Given the molecular complexity of cancers, including the extreme heterogeneity in their genetic makeup, their plasticity, innate or acquired resistance to anticancer drugs, there is an increasing need for the development of single drugs that can effectively and simultaneously target multiple pathological processes to achieve greater antitumor efficacy. In this study we developed a hybrid drug by combining the Cox-2 selective NSAID rofecoxib with a trimethoxy aryl group found in combretastatin A4 (CA4) which is a potent antimicrotubule and anti-angiogenesis agent. Two of methoxy groups of the CA4, were, however, replaced with Iodine in the hybrid drug named KSS-19. KSS-19 was highly potent in killing the tumor cells of various human cancer types at 2-50 nM range. Particularly significant was that KSS-19 was 100 time more potent than CA4 against the highly resistant HT29 colon cancer cells. Various biochemical and immunocytochemical assays revealed that KSS-19 retained the microtubule disrupting effects of compound CA4, including microtubule loss, the formation of aberrant mitotic spindles, and mitotic arrest. It also inhibited the polymerization of purified tubulins *in vitro*. KSS19 retained its capacity to inhibit Cox-2 and elevated the levels of E-Cadherin significantly through NFkB/Snail pathway. Furthermore, KSS-19 potently inhibited the formation of tubes in three-dimensional cultures of the HUVEC (Human umbilical vein cell) at 250 nM with drastic decrease in the tube length and number of junctions. The drug also markedly downregulated the tumor invasion and migration in designated assays. The Cox-2 dependent properties of KSS-19 and its antitumor impact is tested in mouse xenograft model bearing colon cancer and we observed significant regression in tumor volume with no toxic effects on rest of the body organs. The molecular modelling for both targets Tubulin and COX-2 gave promising results strengthening the *in vitro* and *in vivo* results. In summary, the designs of such single molecule hybrid anticancer drugs hold great promise for treatment of colorectal and other cancers.

2033 26-Week Carcinogenesis Study of 1,2-dichloroethane by Dermal Application in CB6F1-TG rasH2 Mice

M. Suguro, T. Numano, M. Kawabe, M. Akiyama, Y. Doi and Y. Mera. *DIMS Institute of Medical Science, Inc., Ichinomiya, Japan.*

It has been reported 1,2-dichloroethane showed the lung carcinogenicity by repeated dermal application to back skin of CD-1(ICR) mice for 440 to 594 days. The present study was conducted to investigate whether it can be detected in a shorter period for the carcinogenic potential of the 1,2-dichloroethane using 26-week carcinogenicity study in rasH2 mice. The back skin of rasH2 mice of both sexes (8 weeks age, 10 mice in each sex per group) were clipped, and 1,2-dichloroethane was applied at the dose of 126 mg/100 µL acetone 3 times/week. And, acetone or 80% ethanol (both 100 µL/mouse) commonly used as vehicle were applied once daily. As positive control, MNU was administered by single intraperitoneal injection at a dose level of 75 mg/kg. Animals were sacrificed at week 26, and histopathological examination was conducted for all organs and gross lesions. In female 1,2-dichloroethane group, 5 animals died after week 17. In gross pathological examination at necropsy, discolored area or nodule of lung were observed in 9 and 10 mice of male and female 1,2-dichloroethane groups, respectively. These lesions were diagnosed as alveolar hyperplasia, adenoma or adenocarcinoma of lung, histopathologically. The incidence of adenoma and adenocarcinoma was 80% and 50% in male mice and 70% and 100% in female mice, respectively, and significant increase was found as compared with the acetone group (0%). In the MNU group, neoplastic lesions were observed in all mice, and the sensitivity of rasH2 mice used in this study was confirmed. In conclusion, lung carcinogenicity of 1,2-dichloroethane could be detected by repeated skin application in rasH2 mice for 26 weeks. On the other hand, acetone and 80% ethanol showed no carcinogenicity in this study. Thus, present results demonstrated that the carcinogenicity of the substance applied skin could be elucidated by dermal application in this 26-week carcinogenesis study using rasH2 mice.

2034 Activation of the Dietary Carcinogens B[a]P and PhIP in LNCaP Prostate Cancer Cells in the Presence of IL-6

O. Graham and N. J. Gooderham. *Department of Surgery and Cancer, Imperial College London, London, United Kingdom.*

Dietary carcinogens are an important risk factor for developing prostate cancer - the most commonly diagnosed cancer in men in the UK and USA. Two important dietary prostate carcinogens are benzo[a]pyrene (B[a]P) and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), pro-carcinogens requiring metabolic activation to their carcinogenic derivatives by the cytochrome P450 (CYP) enzyme family. Inflammation is common in the prostate, with interleukin-6 (IL-6) levels increasing in the serum of prostate cancer patients. In the current study, the metabolic and genotoxic effects of B[a]P, PhIP and IL-6 - alone and in combination - on LNCaP prostate cancer cells were investigated. LNCaP cells were incubated with B[a]P (1nM-10µM), PhIP (10nM-100µM) or IL-6 (30pg/ml-20ng/ml) for various time points up to 72h, and gene expression (qPCR) and genotoxicity (micronucleus assay) were investigated. Both B[a]P and PhIP were found to induce CYP1A1 mRNA expression, whereas only B[a]P significantly induced CYP1A2 expression, with a 250-fold increase from control. B[a]P was found to be genotoxic to LNCaP cells, whereas PhIP was not. Interestingly, in line with recent reports that CYP2E1 expression is controlled by JAK-STAT, PhIP (100µM) induced CYP2E1 expression, with associated induction of JAK2 and STAT3 expression. In contrast IL-6 treatment caused a dose-dependent decrease in CYP2E1 expression, but a concomitant induction of STAT3, suggesting that the induced STAT3 operated outside of the CYP2E1 induction pathway. Treatment with binary mixtures of PhIP (10nM-100µM) and IL-6 (1000pg/ml) resulted in CYP2E1 expression significantly lower than that following treatment with PhIP alone, and mixtures of B[a]P (1nM-10µM) and PhIP (100µM) caused CYP1A2 expression to be significantly higher than after treatment with either B[a]P or PhIP alone. These data suggest that exposure of prostate cells to mixtures of B[a]P and PhIP in an inflammatory environment (e.g. IL-6) alter the expression of CYP enzymes involved in the metabolic activation of these compounds thereby possibly altering their genotoxic potential.

2035 Preventive Effects of Butyrate-Containing Structured Lipids on Experimental Liver Carcinogenesis

J. F. Ortega[1], R. Heidor[1], K. Furtado[1], J. Ract[1], L. Gioieli[1], E. Purgatto[1], A. de Conti[2], I. Pogribny[2] and F. Moreno[1]. [1]*Biochemical and Pharmaceutical Technology, University of São Paulo, São Paulo, Brazil and* [2]*Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR;* [3]*Food and Experimental Nutrition, University of São Paulo, São Paulo, Brazil.*

Emerging evidence indicates that the use of bioactive food components is a promising strategy to prevent the development of liver cancer. Previously, we demonstrated that tributyrin, a butyric acid pro-drug, exhibited a strong chemopreventive activity in experimental liver carcinogenesis. One promising approach in the field of cancer prevention is developing "combination chemopreventive strategies" using agents with different mechanisms of action to simultaneously target multiple cancer-related pathways. In the present study, we investigated a chemopreventive effect of structured lipids (STLs) obtained by enzymatic interesterification of tributyrin, and flax seed oil, a rich source of alpha-linolenic acid, on rat liver carcinogenesis. Treatment of rats subjected to the "resistant hepatocyte" model of hepatocarcinogenesis with STLs during the promotion phase inhibited the development of preneoplastic lesions. This inhibitory effect was associated with the induction of apoptosis, but not with inhibition of cell proliferation. To investigate the underlying mechanisms of chemoprevention mediated by STLs, the expression of genes involved in the liver cancer process were analyzed. Treatment of rats undergoing hepatocarcinogenesis with STLs efficiently reduced expression of major oncogenes Bcl2, Ccnd2, and of genes involved with angiogenesis Pdgfa, Vegfa. Moreover, treatment with STLs increased the expression of critical tumor suppressor genes Cdh13, Socs3 and Fhit. In conclusion, the tumor-suppressing activity of STLs may be attributed to the reduction of expression of oncogenes and induction of expression of tumor suppressor genes at early phases of hepatocarcinogenesis.

2036 No Lung Pathology in Mice from Twelve Months of Exposure to Styrene in the Absence of Cyp2f2 Metabolism

G. Cruzan[4], J. S. Bus[2], M. I. Banton[5], S. S. Sarang[7], R. Waites[6], D. Layko[3] and J. Raymond[1]. *[1]Charles River Laboratories, Frederick, MD; [2]Exponent, Midland, MI; [3]The Hamner Institutes for Health Sciences, Research Triangle Park, NC; [4]ToxWorks, Bridgeton, NJ; [5]HSE, Lyondell Bassell Corporation, Houston, TX; [6]Product Stewardship and Toxicology, SABIC, Mount Vernon, IN and [7]Shell Health, Shell International, Houston, TX.*

Exposure of CD-1 mice to 40 to 160 ppm styrene 6 hours/day 5 days/week for 104 weeks caused increased lung tumors. Lung pathology was seen after 1 week including exfoliation of cells, increased BrdU labeling in terminal bronchioles and decreased staining of Club cells progressing to hyperplasia in terminal bronchioles by 12 months. Lung tumors were observed only at 104 weeks or in mice that died after 78 weeks. Mode of Action studies have demonstrated that in the absence of CYP2F2 metabolism (CYP2F2 knockout mice, KO), there was no acute lung toxicity, no increase in BrdU labeling, and no changes in gene expression in the lungs from styrene exposure. Furthermore, replacing the mouse CYP2F2 with the human CYP2F1, resulted in no effects in the lungs from styrene exposure. The present study is examining the effect of CYP2F2 metabolism of styrene on long-term lung pathology and tumor formation. Male CD-1, C57BL/6 (WT), CYP2F2 KO, and CYP2F2 KO, CYP2F1 transgenic (TG) mice are being exposed to 0 or 120 ppm styrene for up to 104 weeks, with interim terminations at 1, 26, 52, and 78 weeks. After 1, 26 or 52 weeks, degeneration, necrosis, and exfoliation of epithelial cells and/or epithelial hyperplasia in the terminal bronchioles was observed only in CD-1 and WT mice exposed to styrene. No pathologic changes or hyperplasia were present in the terminal bronchioles of KO or TG mice. These data demonstrate that metabolism of styrene by CYP2F2 is required for chronic toxicity and hyperplasia in the lung. Importantly, based on the absence of findings in the TG mice, such effects are not expected in humans exposed to styrene. Completion of the study will determine the effect of CYP2F2 metabolism on styrene-induced mouse lung tumors. Supported by Styrene Information and Research Center (SIRC).

2037 Effect of the Non-genotoxic Carcinogens, Methapyrilene Hydrochloride and Bis-(2-Ethylhexyl) Phthalate (DEHP), on Intracellular Iron Metabolism in Rat Liver

I. Kindrat[1,2], S. Shpyleva[2], T. Chen[2], F. A. Beland[2] and I. P. Pogribny[2]. *[1]National Medical University, Ivano-Frankivsk, Ukraine and [2]NCTR, US FDA, Jefferson, AR.*

Liver, the central detoxification organ and main regulator of systemic iron homeostasis, is prone to damage by xenobiotics. It is well established that hepatotoxicity, especially chronic hepatoxicity, associated with exposure to certain natural and man-made chemicals is a risk factor for fibrosis, cirrhosis, and hepatocellular carcinoma. However, there are limited data regarding the influence of hepatotoxins and hepatocarcinogens on the status of hepatic iron metabolism. In light of this, we investigated the effect of methapyrilene hydrochloride, bis-(2-ethylhexyl) phthalate (DEHP), and usnic acid, chemicals possessing different potentials for liver toxicity and carcinogenicity, on iron metabolism in rat livers. Male Fischer 344 (F344) rats were treated with either methapyrilene at doses 40 and 80 mg/kg body weight (bw)/day or usnic acid at doses 60 and 120 mg/kg bw/day by gavage for 6 weeks, or fed diet containing 1.2% and 2.4% of DEHP for 6 weeks. While treatment of F344 rats with methapyrilene or DEHP resulted in assorted and dose-independent changes in the expression of iron metabolism genes and hepatotoxicity-marker genes, both non-genotoxic carcinogens caused a marked dose-dependent over-expression of the ferritin, light polypeptide (Ftl) gene that codes the main intracellular iron storage protein in the liver. Importantly, there was a significant correlation between the expression of Ftl and apurininic/apyrimidinic endonuclease (Apex1) genes ($r=0.7$; $p<0.001$ and $r=0.95$; $p<0.001$, respectively) in the liver of rats treated with methapyrilene hydrochloride or DEHP. In contrast, exposure of F344 rats to usnic acid, a non-carcinogenic and low hepatotoxicity agent, did not change the expression of hepatotoxicity markers or iron metabolism genes. These results demonstrate an involvement of hepatic iron metabolism dysregulation in the pathogenesis and mechanism of chemical-induced liver toxicity, and suggest that these changes may be sensitive and conventional indicators of potential chemical agent hepatotoxicity.

2038 Using Transcriptomics and Cell Proliferation to Evaluate Mode of Action of Ethylbenzene in Wild-Type, Cyp2f2 Knockout and Cyp2f1 Humanized Mice Exposed for 5 Days

M. E. Andersen[2], G. Cruzan[3], J. S. Bus[1], M. I. Banton[4], S. S. Sarang[5] and M. Black[2]. *[1]Exponent, Midland, MI; [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC; [3]ToxWorks, Bridgeton, NJ; [4]HSE, LyondellBasell, Houston, TX and [5]Shell Health, Shell International, Houston, TX.*

Metabolism of Ethylbenzene (EB) by mouse lung CYP2F2 to cytotoxic metabolite(s) has been postulated as an essential step for mouse lung toxicity and mouse-lung specific tumorigenicity (Sweeney et al., 2015). The purpose of this study was to use whole-lung and liver genomic and cell proliferation analyses to further investigate potential modes of action (MoA) of EB in C57BL/6 wild-type (WT), CYP2F2 knockout (-/-; KO) and CYP2F21 humanized (2F2-KO + 2F1,2A13,2B6-transgenic, TG) male mice. Mice were exposed to 0, 75 or 750 ppm EB 6 hr/day for 5 days. BrdU incorporation measured cell replication in the lungs of 7 mice/group. Five biological replicates of lung and liver tissue for each treatment group of mice were analyzed for relative gene expression using Affymetrix whole genome HT_MG430_PM Titan arrays. In the lungs of WT mice, EB exposure resulted in increased BrdU labeling, but not in lungs of KO or TG mice. There were many fewer genes differentially expressed in the female KO mouse liver than in the female WT mouse (21 versus 4148 at 750 ppm). In the WT female livers there was upregulation of several glutathione-transferases and alterations in multiple CYP450s. The lung response was quite different, with many more genes affected in the KO female lung than in the WT female lung. In general, there were larger numbers of genes altered in the female KO-mice than in the males. The gene expression changes in the female KO mouse lung showed significant enrichment in metabolic processes related to phospholipid and sphingolipid pathways and altered signaling related to Wnt, Notch, various growth factor signaling, cell cycle, and immune response pathways. Compared to the WT mice, there was a tendency for relatively small increases in the numbers of differentially expressed genes in both tissues from the TG mice. Supported by SIRC.

2039 Transcriptional Profiling Indicates That Hypoxia and Not Formation of Glutathione Metabolites Is a Key Mode of Action for Methylene Chloride Lung and Liver Cancer

M. B. Black, P. D. McMullen, S. N. Pendse and M. E. Andersen. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Methylene chloride (dichloromethane; DCM) is metabolized by high affinity oxidation and low affinity glutathione (GSH) conjugation. Because DCM metabolism through the GSH pathway correlated with lung and liver tumor incidence, cancer risk assessments have used GSH pathway products as a dose measure. PBPK models for DCM can track blood levels of DCM, carbon monoxide (CO) from oxidation, and carboxyhemoglobin (HbCO) in mice. We evaluated gene expression in lung and liver from mice exposed to five different air concentrations of DCM for 6hr/day, 5 days/week for 13 weeks (GEO data GSE17933 and GSE18858). The numbers of genes differentially expressed at FDR<0.05 and |FC|>1.5 were 445, 376, 527, 173, and 143 in lung for exposures at 100, 500, 2000, 3000, and 4000 ppm, respectively; the bioassay used 2000 and 4000 ppm. Pathway enrichment changed with increasing DCM exposure. At the lower exposure levels, there was enrichment for cell cycle and mitotic phase genes; at 2000 ppm metabolism of lipids/lipoproteins and downstream GPCR signaling was enriched; at the higher exposures there was enrichment of circadian clock genes. In liver, for the 5 exposure levels, there were changes in 126, 620, 345, 999 and 623 genes. At the three highest exposure levels, metabolism of lipids/lipoproteins, circadian clock, and GPCR signaling were enriched. Importantly, there was no enrichment of oxidative stress, apoptosis or DNA-damage pathways at any DCM exposure level in either tissue. We did see significant changes in expression for 30 CYP-450s. With the PBPK modeling peak HbCO was expected to be 14% of total heme and persist for 15, 20 and 25 hours at the three highest DCM exposure levels. Overall, our studies indicate that the primary response to DCM at tumorigenic exposure levels is due to altered tissue hypoxia (likely from both elevated HbCO and nearly anesthetic levels of inhaled DCM). Sponsored by the Halogenated Solvents Industry Alliance (HSIA) and the European Chlorinated Solvent Association (ECSA).

2040 Benzo(a)pyrene [B(a)p]-Induced Colon Tumorigenesis Is Enhanced by Western Diet Consumption in the Pirc Rat Model: Role of B(a)p Biotransformation

K. L. Harris[1], M. S. Niaz[1], M. K. Washington[2] and A. Ramesh[1]. [1]*Biochemistry & Cancer Biology, Meharry Medical College, Nashville, TN and* [2]*Pathology, Vanderbilt University, Nashville, TN.*

The role of fat content in diet has been linked to the development of several types of cancers. The objective of this study is to determine whether B(a)P-induced colon polyp burden potentiated by Western diet (WD) is mediated by drug metabolizing enzymes (DME) to enhance the effect of the WD. Groups of Polyposis In the Rat Colon (PIRC) kindred type male rats (n = 8) were fed with AIN-76A regular diet (RD) or WD and received 25,50 and 100 µg B(a)P/kg body wt. via oral gavage for 60 days. Rats fed with diets alone, but no B(a)P, served as controls. Following exposure, rats were euthanized; colons, liver and other tissues were retrieved and preserved in 10% formalin for observation of gross pathological changes. Colon and liver samples were analyzed for expression and activities of CYP1A1, CYP1B1, UDPGT, ST and GST drug metabolizing enzymes. Benzo(a)pyrene metabolite concentrations in plasma, colon and liver samples were measured using a HPLC equipped with UV and fluorescence detectors. An increased incidence of adenomas and high grade dysplasia were encountered in rats that were fed with WD compared to RD and controls ($p < 0.05$). The colon tumor counts and sizes were more in B(a)P + WD rats compared to their B(a)P + RD counterparts, and also exhibited a B(a)P dose-response relationship. Adenomas with high grade dysplasia were prominent in B(a)P + WD rats compared to B(a)P + RD rats. Western diet consumption increased metabolic activation and reduced detoxification among rats that were given B(a)P + WD. A marked increase in enzyme expression and B(a)P metabolite concentrations in rats that were administered 100 µg/kg B(a)P + WD ($p < 0.05$) compared to other treatment groups was noted. Our results demonstrate that WD accelerates the development of colon tumors induced by B(a)P, utilizing B(a)P biotransformation pathways. This research was funded by NIH (1F31ESO2407901, 5RO1CA142845-04, 5R25GM059994-11) and Southern Regional Education Board.

2041 Mode of Action (MOA) of Perfluorooctanoic acid (PFOA) Induced Liver and Pancreatic Acinar Cell Tumors in Rats

X. Li, Q. Wu, Z. Wang and J. E. Klaunig. *Environmental Health Science, Indiana University, Bloomington, IN.*

Chronic exposure of PFOA was shown to induce liver, Leydig cell and pancreatic acinar cell tumors (tumor-triad) in rats. The MOA of PFOA induced the liver tumors appears to be predominantly through the activation of PPARα. In contrast, a definitive MOA for the observed pancreatic tumors has not been resolved and as such the current study was performed. Male Sprague Dawley rats were treated with 0, 1, 5 or 15mg/kg PFOA by oral gavage for 7, 14, and 28 days. A dose dependent increase in relative liver weight was found in PFOA treated rats. Hepatic PPARα activation was increased as measured by increased peroxisomal acyl-CoA activity (3-4 fold) and Cyp4a1, Acox1 mRNA (~30 fold) in a dose related manner. Hepatocyte DNA synthesis (BrdU labeling index) also increased in a dose dependent manner at the 7 day exposure time, returning to control levels after 14 and 28 days. In contrast to the liver, no change in relative pancreas weight was seen at any of the dose or treatment times. Pancreatic PPARα (Cyp4a1; Acox1) and PPARγ (Lpl) mediated genes increased 2 to 4 fold over control in the 5 and 15 mg/kg treatment groups at 14 days. No significant changes were found in CAR, PXR, AhR mediated gene expression in the pancreas after PFOA treatment. At the highest PFOA dose studied, an increase in TNFα and IL-6 gene expression was seen. No change was observed in BrdU labeling index in the pancreas after PFOA treatment. Interestingly, Ki-67 labeling index decreased by 65% after 14 days at the highest dose studied. This decrease correlated with an increase in caspase-3 gene expression suggesting an apoptotic process was involved. In summary, we confirmed the role for PPARα receptor in the MOA of PFOA induced liver tumors in rat. While in the pancreas, expression of both PPARα and PPARγ mediated genes was increased. But unlike the liver where DNA synthesis increased in a dose dependent manner, PFOA in the pancreas reduced the Ki-67 labelling that was concomitant with an increase in apoptotic related genes.

2042 Mechanism of 1,3-Dichloropropene (1,3-D) Induced Liver Tumors in Rat

B. A. Elser, X. Li, Z. Wang and J. E. Klaunig. *Indiana University, Bloomington, IN.*

1,3-Dichloropropene is used as a component in some soil fumigants. Chronic oral exposure to 1,3-D was associated with an increased incidence of liver tumors in male rats. Previously, (Toxicol Sci. 2015;143:6-15) we have shown that the 1,3-D induced liver tumors was through a nongenotoxic mode of action (MOA). The present study examined several established nongenotoxic modes of action in the rodent liver including receptor-mediated, cytotoxicity, and oxidative stress. F344 male rats were treated by gavage with 0 (corn oil), 2.5, 12.5, and 25 mg/kg/day 1,3-D for 7 days. Rats treated with phenobarbital (PB) 80 mg/kg/day or beta-naphthoflavone (BNF) 100 mg/kg/day or diethylhexyl phthalate (DEHP) 600 mg/kg/day were used as positive controls for activation of liver nuclear receptors. DNA synthesis was evaluated by immunohistochemistry using bromodeoxyuridine (BrdU). BrdU labeling index was not increased in 1,3-D treated rats. Similarly, no increase was seen in expression levels of cell proliferation related genes (Ccnd1, c-Myc and Ki-67) by 1,3-D treatment. The apoptotic genes FasL, Bid, Bax and Bcl-2 were also not affected by 1,3-D treatment. PB, BNF and DEHP induced significant increase in the CAR/PXR (Cyp2b1 and Cyp3a1), AhR (Cyp1a1), and PPARα (Cyp4a1) target genes, respectively. 1,3-D had no effect on the expression level of these nuclear receptor genes at any dose examined. No evidence for the involvement of cytotoxicity (negative serum AST/ALT; lack of liver necrosis) was observed in 1,3-D treated rats. Similarly, no difference in oxidative stress biomarkers was seen between 1,3-D treatment and control rats. However, Nrf2 gene expression, which regulates the expression of antioxidant proteins, was significantly higher in the 1,3-D high dose group compared with control. In conclusion, although a definitive MOA for 1,3-D induced rat liver tumors was not identified, several established liver tumor modes of action do not appear to be involved in the 1,3-D induced rat hepatic tumors.

2043 Investigations on the Carcinogenic Mechanism of N-Vinyl-2-Pyrrolidone (NVP)

D. Fruth[3], E. Fabian[3], M. Schulz[3], F. I. Berger[3], F. Oesch[2], B. van Ravenzwaay[3] and R. Landsiedel[3]. [1]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany;* [2]*Institute of Toxicology, Johannes Gutenberg Univiersity, Mainz, Germany and* [3]*Product Safety, BASF SE, Ludwigshafen am Rhein, Germany.*

NVP is widely used as a monomer for polyvinylpyrrolidones (PVP) with applications e.g. in food technology and cosmetics or as reactive diluent for radiation (UV) curing adhesives and inks. NVP is a known hepatocarcinogen in rats after inhalative exposure to 5, 10, and 20 ppm (2 yrs) but is negative in the complete genotoxicity testing battery (e.g. Ames, mouse lymphoma, UDS, chromosome aberration, oral micronucleus test (MNT) in mice bone marrow) [1]. To confirm the absence of genotoxicity and to prove the hypothesis of a non-genotoxic mode of action a five day NVP-inhalation study with concentrations of 0, 5, 10, 20 ppm was conducted in Wistar rats (6 animals/ gender/ group, positive control: ethyl methanesulfonate 200 mg/kg bw p.o.). The MNT was carried out in bone marrow and the Comet Assay was performed in lung and liver with and without formamidopyrimidine-glycosylase (FPG) treatment. Additionally liver samples of this study were used to investigate the following possible mechanisms of carcinogenesis: enzyme induction (CYP1A, CYP2B, CYP3A), peroxisome-proliferation (CYP4A, cyanide-insensitive palmitoyl-CoA oxidase) and oxidative stress (glutathione, GSH/GSSG status). At carcinogenic doses NVP was not genotoxic in lung, liver and bone marrow as neither the tail intensity in the Comet Assay nor the number of micronuclei in the MNT was relevantly increased compared to the controls. There was also no detectable influence on the tested enzyme activities as well as on glutathione levels. Based on these data direct genotoxicity, oxidative stress, receptor-binding (AhR, CAR, PXR, PPARα) are not likely mechanisms of NVP-induced hepatocarcinogenicity. 1 Klimisch et al., Food and Chemical Toxicology 1997, 35,1041-1060

2044 Diindolylmethane and Its Halogenated Derivatives Induce Protective Autophagy in Human Prostate Cancer Cells via Induction of Astrocyte Elevated Gene-1 (AEG-1) and Activation of AMP-Activated Protein Kinase (AMPK)

H. Draz[1], A. Goldberg[1], S. H. Safe[2] and J. T. Sanderson[1]. *[1]INRS–Institut Armand-Frappier, Laval, QC, Canada and [2]Veterinary Physiology and Pharmacology, Texas A&M University, Texas, TX.*

We have previously shown that 3,3′-diindolylmethane (DIM) and its halogenated derivatives (ring-DIMs) induce apoptosis and autophagy in human prostate cancer cells. The mechanisms of action of ring-DIMs are not fully understood, but are multifaceted and dependent on halogen substitution pattern. As DIM is an ATPase inhibitor, we hypothesized that DIM and ring-DIMs induce autophagy via activation of AMPK in prostate cancer cells. Autophagic activity was monitored by GFP-LC3B punctuation and LC3BI to LC3BII conversion in LNCaP and C42B prostate cancer cells. Mitochondrial proteomic analysis was done by LC-MS/MS. Protein levels for AMPK, pAMPK, acetyl-CoA carboxylase (ACC), pACC , AEG-1, pULK-1 and β-actin were measured by western blot. AMPK and AEG-1 gene expression was downregulated using siRNA. Apoptosis and necrosis were assessed with Hoechst 33342 and propidium iodide, respectively. DIM and ring-DIMs induced autophagy by increasing LC3BI to LC3BII conversion and LC3B punctuation in LNCaP and C42B cells. DIM and ring-DIMs induced AMPK, ULK-1 and ACC phosphorylation concentration- and time-dependently. Interestingly, DIM, 4,4′ dibromoDIM and 7,7′ dichloroDIM induced the oncogenic protein AEG-1. LC-MS/MS analysis revealed that AEG-1 was recruited to the mitochondria upon ring-DIM treatment. Downregulation of AEG-1 or AMPK significantly inhibited DIM- or ringDIM-induced autophagy. Pretreatment with autophagy inhibitors Baflomycin A1, 3-Methyladenine, the ULK1 inhibitor MRT 67307 or siRNAs targeting AEG-1 or AMPK significantly exacerbated the cytotoxicity of DIM and ring-DIMs. In summary, we identified a novel mechanism for DIM- and ring-DIM-induced protective autophagy, via induction of AEG-1 and activation of AMPK. Our findings could help towards the development of novel drug therapies for prostate cancer that include selective autophagy inhibitors as adjuvants.

2045 Gene Expression Changes Following Repeat Oral Administration of Tetrabromobisphenol A (TBBPA) in Female Wistar Han Rats

S. M. Hall, S. J. Coulter, G. A. Knudsen, J. M. Sanders and L. S. Birnbaum. *Toxicology and Toxicokinetics, NCI at NIEHS, Research Triangle Park, NC.*

Tetrabromobisphenol A (TBBPA) is the most commonly used brominated flame retardant by volume, and human exposure is ubiquitous. A National Toxicology Program 2-year cancer bioassay concluded that TBBPA induces dose-dependent increases in uterine tumors in female Wistar Han rats. Our lab has previously examined the impact of disruption of estrogen signaling pathways on uterine carcinogenesis. To expand on this research, an analysis was undertaken to determine whether TBBPA would induce additional gene expression alterations in uterus and liver tissues. TBBPA (250 mg/kg/day) or vehicle was orally administered for 5 days to female Wistar Han rats. Liver (left lobe) and uterus (distal and proximal) samples were collected at necropsy 24 hours following the fifth administration. Using Gene Set Enrichment Analysis (GSEA), microarray data were evaluated using gene set collections from the Molecular Signatures Database (MSigDB). Results indicated that several pathways involved in immune response (e.g., TLR signaling, T cell receptor signaling) were downregulated in the distal uterus in response to TBBPA but not in the proximal uterus or the liver. In the distal uterus and liver, TBBPA also upregulated several pathways associated with Myc targets, E2F transcription factor targets, and the G2/M checkpoint of the cell cycle. Additionally, TBBPA upregulated genes involved in WNT beta catenin signaling in the distal uterus. In contrast, TBBPA downregulated these same pathways in the proximal uterus. These microarray data are being validated and cross-referenced using RNA-Seq and digital PCR. This work was supported by the Intramural Research Program of the National Cancer Institute at the National Institutes of Health [Project ZIA BC 011476].

2046 Low Doses of DiEthylHexylPhthalate (DEHP) and monoEthylHexylPhthalate (mEHP) Stimulate the Proliferation of Breast Cells

E. Ferraris, E. Kolasa and I. Plante. *INRS - Institut Armand-Frappier, Laval, QC, Canada.*

DEHP, an ubiquitous plasticizer, is considered to be an endocrine disruptor (ED). DEHP and its metabolite, mEHP, have been detected in different human matrices including breast milk. Some studies have shown that mEHP may influence the activity of the peroxysome proliferated-activators (PPARs). These nuclear receptors act as transcription factors. A DEHP exposure could thus modify cell signalling, alter cell proliferation and differentiation, thereby promoting breast cancer. The objective of this study is to determine the consequences of DEHP and mEHP exposure on breast cancer and its progression, and more specifically to identify the mechanisms contributing to the toxicity of this ED. T47D and hTERT-HME1 mammary cells were exposed to environmental doses of DEHP and mEHP for seven days and their proliferation was measured in real time with the xCELLigence system. Increased proliferation was measured in T47D cells for the 10 µM dose of DEHP and 100 pM dose of mEHP. No effects were observed for the hTERT-HME1 cells. Progesterone receptor protein levels were increased in T47D cells exposed to 10 µM of DEHP. Furthermore, an increase in PPARβ/δ protein levels was observed in mEHP-exposed T47D cells for the dose of 10 µM. PPARβ/δ protein levels were also increased in the hTERT-HME1 cells exposed to 100 pM of DEHP and 100 nM of mEHP. Preliminary results of RT-qPCR showed an increase in mRNA levels of genes linked with adipogenesis for the T47D and hTERT-HME1 cell lines. Globally, our results suggest that low doses of DEHP and mEHP can induce tumorigenic processes in the mammary gland. This study will provide a better understanding of the risks associated with DEHP and mEHP exposure and the molecular mechanisms involved in their toxicity. Financed by SRC (IP), FQCS (IP), FRQS (IP, EK) and FUAFI (EF, EK).

2047 Flaxseed Lignans Enriched in Secoisolariciresinol Diglucoside (SDG) Prevent Asbestos-Induced Peritoneal Inflammation in Mice

R. A. Pietrofesa[3], A. Velalopoulou[3], E. Arguiri[3], C. W. Menges[1], J. R. Testa[1], W.-T. Hwang[3], S. M. Albelda[3] and M. Christofidou-Solomidou[3]. *[1]Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA; [2]Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA and [3]Division of Pulmonary, Allergy, and Critical Care Medicine and the Department of Medicine, University of Pennsylvania, Philadelphia, PA.* Sponsor: T. Penning.

Background: Malignant mesothelioma (MM), linked to asbestos exposure, is a highly lethal form of thoracic cancer with a long latency period, high mortality and poor treatment options. Chronic inflammation and oxidative tissue damage caused by asbestos fibers are linked to MM development. Flaxseed lignans, enriched in secoisolariciresinol diglucoside (SDG), have antioxidant, anti-inflammatory and cancer chemopreventive properties. Rationale: As a prelude to chronic chemoprevention studies for MM development, we tested the ability of flaxseed lignan complex (FLC) to prevent acute asbestos-induced inflammation in MM-prone Nf2+/mut mice. Methods: Mice (n=16-17 per group) were placed on control (CTL) or FLC-supplemented diets initiated 7 days prior to a single intraperitoneal bolus of 400 µg of crocidolite asbestos. Three days post-asbestos exposure, mice were evaluated for abdominal inflammation, proinflammatory and profibrogenic cytokine release, WBC gene expression changes, and oxidative and nitrosative stress in peritoneal lavage fluid (PLF). Results: Asbestos-exposed mice fed CTL diet developed acute inflammation, with significant ($p<0.0001$) elevations in PLF WBCs and proinflammatory/profibrogenic cytokines (IL-1ß, IL-6, TNFα, HMGB1 and active TGFß1) relative to baseline (BL) levels. Alternatively, asbestos-exposed FLC-fed mice had a significant ($p<0.0001$) 58% decrease in PLF total WBCs and proinflammatory/profibrogenic cytokine levels relative to CTL-fed mice. PLF WBC gene expression of cytokines (IL-1ß, IL-6, TNFα, HMGB1 and active TGFß1) and cytokine receptors (TNFαR1, and TGFßR1) were also down-regulated by FLC. Importantly, FLC also significantly ($p<0.0001$) blunted asbestos-induced increases in PLF nitrite and MDA levels, indicators of nitrosative and oxidative stress, by 82% and 75% compared to CTL-fed mice, respectively. Conclusion: FLC reduces acute asbestos-induced peritoneal inflammation, and nitrosative and oxidative stress in mice, and may thus prove to be a promising agent in the chemoprevention of MM.

2048 Inhibition of Pim Kinase Sensitizes Acute Myeloid Leukemia Cells with FLT3-ITD to Induction of Apoptosis by Topoisomerase-2 Inhibitors through Increased Oxidative Stress and DNA Damage

K. A. Doshi[1], K. Natarajan[1], A. Tron[2], D. Huszar[2] and M. R. Baer[1]. [1]*University of Maryland Greenebaum Cancer Center, Baltimore, MD and* [2]*Oncology iMED, AstraZeneca, Waltham, MA.*

Fms-like tyrosine kinase-3 receptor internal tandem duplication (FLT3-ITD) mutations are present in acute myeloid leukemia (AML) cells in 30% of patients and are associated with short relapse-free and overall survival. The oncogenic serine/threonine kinase Pim-1 is transcriptionally upregulated downstream of FLT3-ITD and enhances its signaling in a positive feedback loop. We have shown that Pim kinase inhibition sensitizes FLT3-ITD cells to FLT3 inhibitors. Here, we hypothesized that Pim kinase inhibition might also sensitize FLT3-ITD cells to apoptosis induction by chemotherapy drugs. Cytarabine (AraC) and topoisomerase-2 (TOP-2) inhibitors are the chemotherapy drugs commonly used in AML therapy. We found that the Pim kinase inhibitor AZD1208 increased apoptosis induction by TOP-2 inhibitors, including daunorubicin (DNR), mitoxantrone (Mitox) and etoposide (VP-16), as evidenced by increased cells in subG1 phase and increased Annexin V staining. Further, we observed enhanced intrinsic cell death signaling through rapid loss of mitochondrial membrane potential, release of cytochrome c and cleavage of caspase-3 and PARP. However, AZD1208 did not sensitize FLT3-ITD cells to apoptosis induction by AraC. AZD1208 also increased TOP-2 inhibitor-induced apoptosis of FLT3-ITD AML patient samples, but not remission bone marrow cells. Mechanistically, AZD1208 and TOP-2 inhibitors, but not AraC, increased generation of reactive oxygen species (ROS) and induced DNA double-strand breaks, as evidenced by increased γH2AX foci. Pretreatment with the ROS scavenger N-acetyl cysteine (NAC) reduced apoptosis induction by AZD1208 and TOP-2 inhibitors. We conclude that Pim kinase inhibition enhances apoptosis induction of FLT3-ITD cells by TOP-2 inhibitors through increased oxidative stress and DNA damage. Our work supports potential clinical efficacy of combining a Pim kinase inhibitor with TOP-2 inhibitors, but not with AraC, to treat AML with FLT3-ITD.

2049 A Novel Chemoinformatics Approach for Prioritizing Pesticides and Other Carinogenic Hazards for IARC Monographs Evaluation

D. Kumar Barupal, N. Guha, D. Loomis and K. Z. Guyton. *IARC-WHO, Lyon, France.*

Identifying cancer hazards is the first step towards cancer prevention. The IARC Monographs Programme, which has evaluated nearly 1000 agents for carcinogenic potential since 1971, selects agents for hazard identification on the basis of public nomination and expert advice considering suspicion of carcinogenicity and human exposure. A 2014 IARC Advisory Group recommended evaluation of pesticides with high priority. To inform selection among the broad range of pesticides nominated for evaluation, we developed a novel prioritization strategy using chemoinformatics, database integration and automated text mining. We first compiled a comprehensive list of nearly 6000 relevant chemical structures, including from US and EU government databases, ontologies and toxicological databases. We systematically compiled information on the 980 identified pesticide actives, creating chemical similarity network maps that allowed cluster visualization by chemical similarity, class, the public availability of toxicity data, and the number of publications concerning epidemiology, cancer bioassays, and carcinogenic mechanisms. The results informed selection of organophosate, organochlorine and chlorphenoxy pesticides evaluated by the IARC Monographs Programme in March and June 2015. This systematic approach, accounting for chemical similarity and overlaying multiple data sources, can be used by risk assessors as well as researchers to systematize, inform and increase efficiency in selecting and prioritizing agents for hazard identification, risk assessment or further investigation. This strategy could be extended to other outcomes and agents, including occupational carcinogens, drugs, and foods.

2050 Lack of Involvement of Mutagenicity in the Modes of Action Underlying Furan-Induced Hepatocarcinogenesis

D. Hibi, Y. Yokoo, T. Tsuchiya, Y. Ishii, S. Takasu, A. Kijima, K. Ogawa and T. Umemura. *Division of Pathology, National Institute of Health Sciences, Tokyo, Japan.*

Furan has been used as an intermediate in chemical-manufacturing industries and is unintentionally generated during heating of various foods. Although furan is a hepatotoxic and hepatocarcinogenic compound, the tumorigenic modes of action (MOAs) remain unclear because of equivocal results in mutagenicity tests. In the present study, to elucidate the possible MOAs underlying furan-induced hepatocarcinogenesis, comprehensive medium-term analysis was conducted using gpt delta rats treated with furan at carcinogenic doses for 13 weeks. Reporter gene mutation analysis, quantitative analysis of glutathione S-transferase placental form (GST-P)-positive foci, and measurements of cell proliferation-related markers were performed in liver samples. From these analyses, foci of hepatocellular alterations, apoptotic hepatocytes, oval cell proliferation, and cholangiofibrosis were observed in the livers of furan-treated groups. The frequencies of gpt and Spi- mutants derived mainly from point and deletion mutations, respectively, were not changed, and there were no furan-specific gpt mutations in furan-treated rats. The number and area of GST-P-positive foci and the ratio of PCNA-positive hepatocytes were significantly increased in furan-treated rats. Additionally, we found significant increases in cyclin D1 and cyclin E1 mRNA levels. However, in the absence of phosphorylation of extracellular signal-regulated kinase (ERK1/2), it is unlikely that the mitogen-activated protein kinase (MAPK)/ERK pathway contributes to furan-induced cell proliferation. Thus, our results showing that furan exposure was able to increase the number of preneoplastic lesions in hepatocytes, but not induce *in vivo* mutagenicity at the target organ under the current experimental conditions strongly suggested that genotoxic mechanism were not involved in the MOA underlying furan-induced hepatocarcinogenesis. Alternatively, cell proliferation may play a crucial role in furan-induced hepatocarcinogenesis; however, the specific signaling pathways involved in this process were not determined.

2051 Characterization of Histone Modification and Transcriptional Complexity during MMAsIII-Induced UROtsa Cell Malignant Transformation

J. Zhu[2], X. Chen[2], J. Wang[1], X. Yan[2], M. Buck[1], J. R. Olson[3] and X. Ren[2,3]. [1]*Department of Biochemistry, The State University of New York, Buffalo, NY;* [2]*Department of Epidemiology and Environmental Health, The State University of New York, Buffalo, NY and* [3]*Department of Pharmacology and Toxicology, The State University of New York, Buffalo, NY.*

Arsenic exposure is a worldwide public health problem, and an established cause of a variety of cancers, including bladder cancer (BCa). Our previous studies showed that exposure of human uroepithelium cells (UROtsa cells) to monomethylarsonous acid (MMAsIII), the toxic metabolite of arsenic, alters acetylation level of histone H3 and H4 through the disruption of balanced activity between histone deacetylases (HDACs) and histone acetyltransferases (HATs), whereas an HDAC inhibitor can reverse these effects caused by MMAIII and block MMAIII-induced cell malignant transformation, suggesting the the involvement of epigenetic mechanisms, e.g., post-translational modification of histones in arsenic-induced carcinogenesis. Among these lysine residues in Histone 3 and 4, we observed a significant increase of H3K18 acetylation (H3K18ac) in arsenic-induced malignant transformed UROtsa cells vs. in control cells, which was time-dependent. We thus mapped and identified regions in the genome where H3K18ac are changing during the time course of chronic MMAIII exposure. Transcriptomes were also generated by RNA-seq from these same MMAIII-treated UROtsa cells. Utilizing the Galaxy package Cistrome and other bioinformatics tools (e.g. pathway analysis software), it enable us to examine how the arsenic-induced changes in gene expression are regulated by the altered histone acetylation (H3K18ac) during the process of arsenic-induced malignant transformation. These results provide insights into our understanding the relationship between MMAIII-induced changes in histone acetylation and gene expression and the carcinogenesis induced by arsenic. We also expect to identify the inappropriate targeting of regulatory regions of genomic DNA by H3K18ac and the resulted aberrant gene expression, which could help to develop potential novel biomarkers of arsenic carcinogenicity. (This work was supported by NIEHS grant R01ES022629 and R21ES022329 to X.R.).

2052 Association of Single Nucleotide Polymorphism in C10orf32-As3MT with Arsenic Induced Skin Lesions and Skin Cancers

A. Giri[2], N. Das[1] and P. Bhattacharjee[3]. [1]*CSIR-Indian Institute of Chemical Biology, Kolkata, India;* [2]*Queens College, City University of New York, Flushing, NY and* [3]*Department of Environmental Science, University of Calcutta, Kolkata, India.* Sponsor: J. Yourick.

More than 150 million people around the world are drinking arsenic contaminated water much above the WHO permissible limit (10µg/l). In West Bengal, India more that 26 million people in 9 districts are exposed to arsenic through drinking water. Arsenic induced a number of dermatological and non-dermatological health problems, including multi-organ disorders and cancer. Previous studies indicates that genetic variants play an important role in arsenic induced skin lesions. Arsenic methyltransferase (As3MT) is a key enzyme of the arsenic biotransformation pathway, which catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to trivalent arsenicals, resulting in the production of methylated arsenicals (MMAs and DMAs). Five single nucleotide polymorphisms (SNPs) within the C10orf32-As3MT region, which is a read-through transcription between the neighboring C10orf32 (chromosome 10 open reading frame 32) and AS3MT (arsenic, +3 oxidation state, methyltransferase) genes, was associated with arsenic-induced skin lesion, identified by GWAS study recently in Bangladesh population. The said region is a candidate for nonsense-mediated mRNA decay (NMD) and do not synthesis protein, however the significant association with arsenicosis is quite interesting and warrants further studies. Our objective is to find out the association of these identified SNPS with arsenicosis (with characteristic skin lesions and skin cancers) in our population. A total of 315 arsenic exposed individuals were recruited from Murshidabad district of West Bengal, India in this study, of which 157 individuals had characteristic arsenic-induced skin lesions and skin cancers (cases) and 158 individuals were without any skin lesion (controls). Among the 5 SNPs (rs9527, rs4290163, rs4919694, rs11191527 and rs11191659), only the G to A polymorphism (rs9527) having at least with one minor allele 'A' was found to be significantly higher in cases compared to controls, implying increased risk toward the development of skin lesions [OR = 3.332, 95% CI: 1.81-6.14; p-value < 0.0001]. Hence, it can be concluded that the G>A change of rs9527 imparts almost three times risk in arsenicosis patients in West Bengal.

2053 Application of the Bhas 42 Cell Transformation Assay for the Assessment of Electronic Cigarettes

D. Breheny[3], O. Oke[3], O. M. Camacho[3], M. D. Gaca[3], K. Pant[1], S. W. Bruce[1], M. K. Laforce[1] and N. Celestin[1]. [1]*Bioreliance, Rockville, MD;* [2]*Computational Tools and Statistics, British American Tobacco, Southampton, United Kingdom and* [3]*Pre-Clinical Assessment, British American Tobacco, Southampton, United Kingdom.*

In vitro cell transformation assays (CTA) are used to assess the carcinogenic potential of chemicals and complex mixtures. They can detect non-genotoxic as well as genotoxic carcinogens. The Bhas 42 CTA has been developed with both initiation and promotion protocols to distinguish between these two classes of carcinogens. Cigarette smoke contains both genotoxic and non-genotoxic carcinogens. It has been shown to act as a tumour promoter *in vivo* and to be positive in the Bhas 42 promotion assay. We have developed a suite of *in vitro* assays to allow us to compare the relative biological effects of new categories of tobacco and nicotine delivery products with those of traditional cigarettes. The Bhas 42 promotion assay has been included in this test battery to provide an *in vitro* alternative for detecting tumour promoters. The activity of a commercially available e-cigarette (Vype ePen) was compared to that of a reference combustible cigarette (3R4F) in the Bhas 42 promotion protocol. Stock solutions of 24 mg/mL particulate matter (PM) were prepared from both products using the Health Canada Intense (HCI) regime. PM was tested up to a maximum concentration of 120 µg/mL (n=3). 3R4F PM produced a clear concentration-dependent response and induced significantly higher numbers of foci than control treatment at all concentrations (Dunnett's test; p<0.0001). The overall activity of PM from the e-cigarette was found not to differ significantly from a DMSO control at any concentration tested. A Generalised Linear Model with log-link function showed that the slopes of the responses across the concentrations were significantly different between products (p<0.0001). This study shows that the Bhas 42 promotion assay can distinguish between tobacco and nicotine products, and could be included as part of a weight of evidence package for comparative risk assessment of tobacco and nicotine based products.

2054 Dmav in Drinking Water Activated Nf-κB Signal Pathway in Bladder Epithelial Cells and Increased Il-1β Level in Urine of Rats

S. Xi and S. Cao. *Department of Occupational and Environmental Health, School of Public Health, China Medical University, Shenyang, China.* Sponsor: J. Pi.

Dimethylarsenic acid (DMAV) is the main product of arsenic methylation metabolism *in vivo*, and is rat bladder carcinogen and tumor promoting agent. Chronic inflammation, bladder epithelium lesions and proliferation might be the basic process of the chronic toxicity effects in DMAV-treated rats. Our previous study showed that DMAV increased the expressions of proliferation factors in bladder urothelium and elevated TGF-β1 secretion in the urine of rats. NF-κB could link inflammation with tumor progression and play a functional role in inflammation and tumorigenesis. NF-κB is activated by a variety of stimuli including growth factors, cytokines, inflammatory agents, pharmacological agents, carcinogens, and stress. In this study, we measured the expressions of mRNA and proteins of NF-κB pathway members, IKKα, IKKβ, p65 and p50 in rat bladder epithelium by qRT-PCR and immunohistochemical analysis after rats receiving drinking water containing 100 and 200 ppm DMAV for 10 weeks. Urine level of IL-1β was determined by ELISA test. We found that DMAV dramatically increased the mRNA levels of NF-κB p50 and IKKα in the bladder epithelium of rats compared to the control group. Immunohistochemical examinations showed that DMAV increased immunoreactivities of IKKα, IKKβ and phospho-NF-κB p50 in the cytoplasm and phospho-NF-κB p50 and p65 in nuclear of rat urothelial cells. In addition, DMAV treated rats exhibited significantly increased IL-1β secretion in urine. These data suggest that DMAV could activate NF-κB signal pathway. Our precious study found DMA elevated expressions of proliferation factors in bladder epithelium and TGF-β level in urine. Together, the possible mechanism of DMAV on bladder epithelium was that DMAV increased expressions of TGF-β and IL-1β, which mediates activation of NF-κB to promote cell proliferation.

2055 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Treatment in Adulthood Causes Urethral Cancer in *Nkx3.1+/-; Pten+/-* Mice

R. W. Moore, A. J. Schneider, T.-M. Lin and R. E. Peterson. *School of Pharmacy, University of Wisconsin-Madison, Madison, WI.*

Exposure to the TCDD-contaminated herbicide mixture Agent Orange has been associated, controversially, with increased prostate cancer risk in Vietnam veterans. Our objective was to develop an animal model for this possible human health problem. We dosed Nkx3.1+/-; CMV-Cre+/Y; Pten-lox+/- C57BL/6J mice with corn oil or TCDD (5 µg/kg loading dose; 1.8 µg/kg weekly maintenance dose, po) starting at 8 weeks of age. Mice (N = 80/treatment) were necropsied at 59 weeks of age or earlier when necessary. TCDD slightly decreased body weight and reduced survival. Numerous pathologies, including lymphomas, were seen in both treatment groups. Ventral and dorsolateral prostates were often enlarged, especially in TCDD-treated mice. Grossly visible prostate tumors and/or enlarged urethras were found in some TCDD- but no corn oil-treated mice. Microscopically, more than half the TCDD-treated mice had carcinoma in the urethra, ventral prostate, and/or dorsolateral prostate (anterior prostates were not examined), while no carcinomas were found in control mice. These lesions are not adenocarcinomas, which Nkx3.1+/-; Pten+/- mice on a mixed genetic background (the basis for our choice of animals) are reported to develop. Instead, they appear to be urothelial carcinomas. Prostatic intraepithelial neoplasia (PIN) scores were significantly lower in prostates from TCDD-treated than corn oil-treated mice, while TCDD increased stromal fibrosis in the prostate. Thus, while we have demonstrated that TCDD treatment in adulthood can cause cancer in the prostate, these cancers most likely spread to the prostate from the urethra, and they are clearly not adenocarcinoma of the prostate, the predominant prostate cancer type in humans. Supported by NIH Grant ES001332.

2056 Piperlongumine Targets Sp Transcription Factors in Cancer Cells

K. B. Karki[2], U. H. Jin[2], S. Safe[2], G. Chadalapaka[2] and I. Jutooru[1]. [1]*Covance,Inc, Madison, WI and* [2]*VTPP, Texas A&M University, College Station, TX.*

Piperlongumine (PL) is a natural product isolated from the plant species Piper Longum L that was identified in a cell-based small molecule screening assay for anticancer agents that selectively kill cancer vs non-cancer cells. Piperlongumine enhances cellular stress by induction of ROS and we investigated the effects of PL on ROS-dependent down-

regulation of specificity protein (Sp) transcription Sp1, Sp3 and Sp4 and pro-oncogenic Sp-regulated gene products. Preliminary studies show that 5, 10 and 15 μM PL inhibited growth of Panc-1 pancreatic, A549 lung, 786-O kidney and SKBR3 breast cancer cells after treatment for 24 or 48 hours and EC50 values for growth inhibition were 10, 21, 15, 15 (24 hours) and 14, 10, 14, 11 μM (48 hours) respectively. These same cell lines were also treated with PL plus the antioxidant glutathione (GSH) and growth inhibition was attenuated at all concentration of PL indicating that piperlongumine-induced ROS was the major induced cytotoxic response. Treatment of the cancer cell lines with 10 μM PL for 24 or 48 hours also downregulated expression of Sp1, Sp3 and Sp4 proteins and co-treatment with GSH reversed this response. PL also downregualted expression of Sp-regulated genes including survival (survivin, bcl-2) growth promoting (cyclin D1, epidermal growth receptor [EGFR]) and angiogenic (vascular epithelial growth factor [VEGF]) genes in several different cancer cell lines. PL also decreases expression of Myc and other Myc regulated genes including miR-17,miR-20a and miR-27a and these effects were also attenuated in cells co-treatment with PL plus GSH. Current studies are investigating the role of PL in modulating upstream pathways required for Sp downregulation and this includes ROS-dependent epigenetic repression of cMyc, downregualtion of miR-27a and miR-20a/miR-17 and induction of the transcriptional repressors ZBTB10, ZBTB34 and ZBTB4 in cancer cells. Our results demonstrate that the selective ROS-dependent cytotoxicity of PL is due, in part, to downregulation of Sp proteins and pro-oncogenic Sp-regulated genes

2057 Perinatal Exposure to the Phthalate Plasticizer DEHP Alters Mammary Glands Development in Rats

E. Kolasa[1], B. Robaire[2] and I. Plante[1]. *[1]INRS - Institut Armand Frappier, Laval, QC, Canada and [2]Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada.*

Phthalates are plasticizers widely found in many daily life products, such as food packages or cosmetics. As a consequence, humans are chronically exposed to these contaminants that can be detected in various human matrices, including blood serum and breast milk. Phthalates are known to be endocrine disruptors, and represent a growing concern in toxicology. They have been linked to multiple adverse effects, such as fertility disorders or metabolic diseases, but they could also be responsible for some cancers. Epidemiological studies have suggested that these plasticizers could increase the risk of developing breast cancer. However, their effects on mammary gland development remain poorly understood. To investigate this issue, female rats were exposed to di(2-ethylhexyl) phthalate (DEHP) during pregnancy and lactation. Female pups mammary glands were collected and analyzed at different key stages of development: post-natal day 3 (PND3, or newborn), PND21 (weaning), PND46 (puberty), and PND90 (adults). Results showed that perinatal exposure to DEHP increases mammary gland weight in adults, without affecting general epithelium development, as ductal elongation measurements were similar for all groups. Microarrays and gene set enrichment analysis showed that adipogenesis related genes are upregulated in mammary gland of DEHP treated animals. Moreover, *in silico* analyses predicted that the PPAR (peroxisome proliferator-activated receptor) pathway could be a major regulator involved in the observed effects. qPCR and histology analysis are ongoing to confirm these data. In conclusion, this study suggested that gestational and lactational exposure to DEHP could increase breast cancer risk by inducing adipogenesis-linked disorders. This work will provide not only a better hazard assessment of phthalates, but also a better understanding of the signaling pathways involved in their toxicity, and in breast cancer in general. Funded by CRS (IP), FQCS (IP), CIHR (BR) and FRQS (IP, EK).

2058 Relative Potency of Mono-Methylated Chrysenes as Ligands in an Aryl Hydrocarbon Receptor (AhR) Signaling Assay

A. Y. Alqassim, J. Cuccia and C. Miller. *Global Environmental Health Sciences, Tulane University, New Orleans, LA.*

Polycyclic aromatic hydrocarbons (PAHs) comprise a family of abundant and ubiquitous chemicals that are present in the environment. The chrysenes are a subgroup of PAHs that are mutagenic, carcinogenic, and environmentally persistent. Most toxicity studies have been directed toward identifying the effects of chrysene itself, but less is known about the mono-methylated forms. The methylated chrysenes are more predominant in fresh and aged crude oil samples, so characterizing their effects will help in understanding the toxicity of oil spills. Chrysenes are activating ligands of the aryl hydrocarbon receptor (AhR). Ligand activated signaling through AhR is likely an initial step in the toxic pathway that is mediated by chrysenes. We used a recombinant humanized yeast reporter system to systematically characterize the differential ability of all six mono-methylchrysenes to activate human AhR signaling. We identified 4-methylchrysene as the most potent activator, having ten-fold or greater activity than the parent chrysene and other mono-methylated chrysenes. Previous studies have shown that PAH ring structures and methylation conferred different signaling potentials for AhR activation. The differential effects of these mono-methylchrysenes on AhR activation may relate to toxic potential of these compounds.

2059 Common Jamaican Herbal Medicine, Bizzy Nut, Inhibits Cell Cycle in Prostate Cancer Cells

A. Chumbow, W. Gray and O. Phillip. *Southern University and A&M College, Baton Rouge, LA.*

Prostate cancer development is dependent on androgens, and the majority of patients respond to androgen ablation. However, virtually every patient will develop hormone-resistant prostate cancer and no longer respond to androgen deprivation therapy (ADT). Biz-2 (the common Jamaican herbal medicine) Kola acuminate elicited anti-proliferative effects in both hormone-responsive (LNCaP cells) and hormone-resistant (DU145 cells) prostate cancer cells. In this study we determined the cytotoxic efficacy of Biz-2 in hormone-responsive (LNCaP cells), the hormone-resistant (DU145 cells) and a normal transformed (RWPE-1) prostate cells. We determined growth inhibitory parameters (GI50, TGI and LC50) over 24hrs to 96 hrs using the MTT assay and cell count. Biz-2 was found to inhibit the proliferation of all tested cell lines in a concentration-dependent and time-dependent manner. Both cancer cells showed significantly higher cytostatic and cytocidal (TGI 230ppm in DU145 verses 860ppm in RWPE-1 and 156ppm in LNCaP verses 860ppm in WRPE-1) as compared to the normal non-transformed RWPE-1. To begin to understand the mechanism of Biz-2 anti-proliferative activity, we used flow cytometry to determine where in the cell-cycle Biz-2 induced proliferation blockage. Analysis of untreated synchronized LNCaP cells by flow cytometry shows that 63% of the cells were in the Gi/Go, and 36% in the G2/M phases of the cell cycle. Upon treatment with Biz-2, over 95% of the cells were in the Gi/Go phases. When these experiments were performed in DU-145 we observed an accumulation of cells in the G2/M phases of the cell cycle. Both western blot and RT-PCR showed a down regulation in the levels of the cell cycle protein. Our results suggest that the mechanism of action of Biz-2 in androgen-dependent and androgen-independent prostate cancer cells is different.

2060 Role of P450 in Hepatotoxicity and Proliferation in Bile Duct Induced by Exposure to 1,2-dichloropropane in Mice

X. Zhang[3,4], C. Zong[3,4], L. Zhang[3,4], E. Garner[1], C. Huang[3], W. Wu[3], J. Chang[3], T. Sakurai[4], S. Ichihara[2] and G. Ichihara[3,4]. *[1]Lovelace Respiratory Reseach Institute, Albuquerque, NM; [2]Mie University, Tsu, Japan; [3]Nagoya University Graduate School of Medicine, Nagoya, Japan and [4]Tokyo University of Science, Noda, Japan.*

Introduction: 1,2-Dichloropropane (1,2-DCP) has been used as an intermediate in production for other chemicals and as a paint remover. IARC reclassified it as carcinogenic to humans (Group 1) according to the epidemiological studies of cholangiocarcinoma among offset color proof-printing workers exposed to 1,2-DCP in Japan. The present study investigated hepatotoxicity and proliferation in the bile duct induced by exposure to 1,2-DCP and role of P450 in its toxicity by inhibiting P450 activity using 1-aminobenzotriazole (1-ABT). Methods: 42 male C57BL/6JJcl mice were randomly divided into 7 groups of six each. Four groups of mice administrated subcutaneously with 1-ABT at 50mg/kg were exposed to 1,2-DCP at 0, 50, 250 and 1250 ppm, respectively via inhalation route, 8hs per day for 4 weeks. The other three groups administered with saline were exposed to 1,2-DCP at 0, 50 and 250 ppm, respectively. Organ samples were collected under anesthesia at the end of the experiment. BrdU was injected intraperitoneally to the mice one hour prior to dissection for observation of proliferation in bile duct epithelial cells. Results: Serious hepatic pathological changes were found in the groups of 1250 ppm 1,2-DCP with 1-ABT injection and 250 ppm 1,2-DCP without 1-ABT injection, including massive necrosis, inflammation, and hepatocyte degeneration. BrdU labeling index tended to increase with exposure levels of 1,2-DCP in the groups without 1-ABT treatment, but this increase was suppressed by administration with 1-ABT. Conclusions: 1-ABT could reduce liver damage induced by 1,2-DCP exposure, indicating that P450 plays an important role in the hepatotoxicity of 1,2-DCP. P450-mediated metabolism of 1,2-DCP might contribute to proliferation in bile duct epithelial cells induced by exposure to 1,2-DCP.

2061 Hypoxia-Inducible Factor1 alpha (HIF-1α) Associated with CpG Demethylation May Regulate the Level of Mir-194-1 through Promoter Activity under the Hypoxia Conditions

Q. Kong, X.-S. Chen and P. Xu. *Key Laboratory of Gene Engineering of Education Ministry, Sun Yat-Sen University, Guang Zhou, China.* Sponsor: X.-F. Ren.

Accumulating evidences suggested that hypoxia-inducible factor 1α (HIF-1α) can regulate numerous miRNAs and as a key regulator of the transcriptional response to hypoxia. In this study, we analyzed the multiple HIF-1α-binding sites in the miR-194-1 promoter region through a bioinformatic analysis. Dual-Luciferase and the CHIP assays confirmed the specific HIF-1α-binding sites. Further, we demonstrated that different truncated of HIF-1α-binding sites in the miR-194-1 promoter relieved transcriptional suppression levels after hypoxia-induction in cultured cells. Moreover, we found that the miR-194-1 promoter harbors several CpG dinucleotide located in the hypoxia response element. Dual-Luciferase assays with differentially methylated reporter constructs indicated that methylation in the miR-194-1 promoter containing CpG sites remarkably decreased transcriptional activity compared to that in unmethylated constructs. In prostate cancer, we found frequent aberrant demethylation of these elements resulting inter-individual differences between miR-194 and HIF-1α levels. Our results provide an insight into the complexity of cancer-associated CpG demethylation augments hypoxia-induced effects by regulation of HIF-1α.

2062 Increased Use of Mechanistic Data in IARC Monographs

L. Benbrahim-Tallaa. *IARC Monographs, International Agency for Research on Cancer, Lyon Cedex 08, France.*

The IARC Monographs identify environmental factors that can increase the risk of human cancer, based on an integrated evaluation of evidence for carcinogenicity in humans and experimental animals, and of carcinogenic mechanisms. Here we review some recent evaluations that were influenced by strong mechanistic evidence, and highlight types of evidence influential in classifications of cancer hazard. 2,3,7,8-Tetrachlorodibenzo-para-dioxin (TCDD) was first classified in Group 1 (carcinogenic to humans) in 1997 based on sufficient evidence in animals and mechanistic considerations, and later confirmed by sufficient evidence in exposed humans in 2012. Subsequently, 2,3,4,7,8-pentachlorodibenzofuran and 3,3',4,4',5-pentachlorobiphenyl were classified in Group 1 on the basis of similarity to TCDD, being carcinogenic in animals via the same AhR-mediated mechanism. Evidence for genotoxicity has also been the basis for mechanistic upgrades. In 2014, 1,3-propane sultone was classified as probably carcinogenic to humans (Group 2A), based on inadequate evidence in humans and sufficient evidence in experimental animals with a mechanistic upgrade supported by a strong evidence for direct genotoxicity. Additionally, evidence of metabolism to reactive moieties has been influential in cancer hazard classifications. One recent example is in the classification of dichloromethane (DCM) in 2014 as probably carcinogenic to humans (Group 2A). The classification was based on limited evidence in humans for biliary tract cancer and non-Hodgkin lymphoma and sufficient evidence in experimental animals. The WG also took into account the strong evidence that DCM metabolism via glutathione-S-transferase T1 (GSTT1) leads to the formation of reactive metabolites, that GSTT1 activity is strongly associated with genotoxicity of DCM *in vitro* and *in vivo*, and that GSTT1-mediated metabolism of DCM does occur in humans. Mechanistic information, including receptor activation, direct genotoxicity and evidence on metabolism to reactive intermediates can thus provide early, robust evidence for carcinogenicity.

2063 Ethyl Acrylate Not Mutagenic in an *In Vivo* gpt delta Transgenic Mouse Assay

R. Ellis-Hutchings[5], K. Wiench[2], S. Murphy[1], S. Masumori[4] and M. Hayashi[3]. [1]*Arkema, Inc., King of Prussia, PA;* [2]*BASF SE, Ludwigshafen, Germany;* [3]*Makoto International Consulting, Ebina, Japan;* [4]*Public Interest Incorporated Foundation Biosafety Research Center, Shizuoka, Japan and* [5]*The Dow Chemical Company, Midland, MI.*

Ethyl acrylate (EA) is used in various industries as an intermediate in the production of emulsion-based polymers. The major use of EA is in the manufacturing of acrylic resins, which are then used in paint formulations, industrial coatings, and latex products. EA was first classified as reasonably anticipated to be a carcinogen by the International Agency for Research on Cancer (IARC, 1986) and then in the NTP Fifth Annual Report on Carcinogens (1989) based upon a gavage study resulting in dose-related forestomach benign and malignant neoplasms in rats and mice. In genotoxicity tests, EA induced DNA damage including chromosomal aberrations and gene/point mutations *in vitro*. When tested *in vivo*, EA was found to be non-mutagenic in systems measuring both the induction of chromosomal damage and induction of DNA damage. In this study, the gpt transgenic mouse (gpt delta mouse) model was used to clarify whether mutagenic mechanisms are involved in EA-induced carcinogenesis according to the OECD guideline 488. Male gpt delta mice were subjected to repeated oral gavage administration of EA at dose levels of 0, 8, 20, or 50 mg/kg/day for 28 days. Based on the dose range-finding study results using C57BL/6JJmsSlc mice, which is the recipient strain of gpt delta, a dosage of 50 mg/kg/day was selected as the high dose as this dose was without a potentially confounding inflammatory response that was observed at 100 and 200 mg/kg/day. Mutant frequency in the liver and forestomach of EA treated mice did not significantly increase from the negative control. Benzo[a]pyrene, used as a positive control, induced point mutation and deletion at a frequency 7-fold and 4-fold higher in the liver and 4-fold and 8-fold higher in the forestomach, respectively than those of the control. Under the conditions of this study, the data support Ethyl acrylate as not a mutagen in the forestomach of mice suggesting a non-mutagenic mechanism for tumor formation in the long-term gavage study.

2064 Overexpression of Histone H3.1 Facilitates Cell Migration

Y. Liu, H. Sun, T. Kluz, F. Wu, X. Zhang, D. Chen, A. Munoz, T. Des Marais, Q. Chen, J. Brocato, C. Jin and M. Costa. *Environmental Medicine, New York University School of Medicine, New York, NY.*

Canonical histone proteins are part of chromosome structure, whose expression is cell cycle dependent with a peak during the S phase. They are also unique for a stem-loop-binding protein (SLBP) structure at the 3' end of their messenger RNA (mRNA) instead of a poly (A) tail. It has been reported that decreased SLBP level and increased polyadenylation of histone H3.1 mRNA is induced by arsenic exposure and this effect may contribute to arsenic carcinogenesis. In order to further study this relationship, we established BEAS-2B stable cell lines that overexpress histone H3.1 in polyadenylated form. These cells exhibited a cell cycle- independent accumulation of polyadenylated H3.1 mRNA. The results from scratch wound-healing assay indicated that overexpressing polyadenylated H3.1 in BEAS- 2B cells promoted cell migration. Further RNA-seq analysis revealed an enrichment of genes that are involved in cell migration and invasion. These results, with our previous *in vitro* and *in vivo* data, strongly suggest a potential causal relationship between polyadenylation of histone H3.1 mRNA and its carcinogenic effects in cells. We also found out that histone H3.3 proteins, which differ from histone H3.1 with only 4 amino acids, do not demonstrate increased cell proliferation or migration when overexpressed in BEAS-2B cells, nor do they lead to tumor formation when injected subcutaneously into the nude mice.

2065 Targeted Disruption of T-Cell Protein Tyrosine Phosphatase in Mouse Epidermis Promotes Chemically-Induced Skin Carcinogenesis

H. Lee[3], M. Kim[3], L. D. Morales[3], A. K. Vazquez[3], T. J. Slaga[2], J. DiGiovanni[1] and D. J. Kim[3]. [1]*University of Texas at Austin, Austin, TX;* [2]*University of Texas Health Science Center at San Antonio, San Antonio, TX and* [3]*University of Texas Rio Grande Valley, Edinburg, TX.*

Environmental factors such as chemical toxicants contribute to the development of skin cancer by creating mutations in housekeeping genes and proto-oncogenes which disrupt intracellular signaling mechanisms. One vital signaling mechanism is tyrosine phosphorylation signaling. Phosphotyrosine signaling is regulated by the counter-activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Recently we reported TC-PTP (PTPN2) is critical regulator of STAT3 signaling in mouse keratinocytes. To assess the role of TC-PTP in skin carcinogenesis, we generated epidermal-specific TC-PTP-deficient (K14Cre. PTPN2fl/fl) transgenic mice. Loss of TC-PTP led to a desensitization to tumor initiator 7,12-dimethylbenz[a]anthracene (DMBA)-induced apoptosis both *in vivo* epidermis and *in vitro* keratinocytes. TC-PTP deficiency in epidermis resulted in a significant increase in epidermal thickness and hyperproliferation (assessed by bromodeoxyuridine (BrdU) labeling index) compared to control mice following treatment with the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). Consistently, primary keratinocytes derived from TC-PTP-deficient mice showed a faster growth rate. To further investigate the role of TC-PTP in skin carcinogenesis, TC-PTP-deficient mice were subjected to two-stage skin carcinogenesis analysis. TC-PTP-deficient mice showed a shortened la-

tency of tumor development and significantly increased numbers of tumors compared to control mice, demonstrating TC-PTP deficiency predisposes mice to skin carcinogenesis. The data reveals TC-PTP may be a novel potential target for the prevention of skin cancer due to its role in the regulation of proliferation and apoptosis in epidermis.

2066 Specificity Protein (Sp) Transcription Factors as Non-Oncogene Addiction Genes in Cancer Cells

E. Hedrick[2], Y. Cheng[2], U.-H. Jin[2], K. Kim[3] and S. Safe[2]. *[1]Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX; [2]Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX and [3]Environmental Health, University of Cincinnati, College Station, OH.*

Specificity protein (Sp) transcription factor (TF) Sp1 is overexpressed in multiple tumors and is a negative prognostic factor for patient survival. Sp1 and also Sp3 and Sp4 are highly expressed in cancer cells and in this study, we have used results of RNA interference (RNAi) to demonstrate that the three TFs individually play a role in cancer cell growth, survival and migration/invasion in SKBR3 and MDA-MB-231 breast, 786-0 kidney, L3.6pL, PANC1,Miapaca2 pancreatic, A549 lung and SW480 colon cancer cell lines. Surprisingly, individual knockdown of Sp1, Sp3, or Sp4 which resulted in inhibition of cell growth and migration and induction of apoptosis, compensation by the other expressed Sp proteins was observed. Moreover, tumor growth in athymic nude mice bearing L3.6pL pancreatic cancer cells as xenografts was significantly attenuated in cells were Sp1, Sp3, and Sp4 was combinatorally depleted or in cells depleted of Sp1 alone. Ingenuity Pathway Analysis (IPA) of changes in gene expression in Panc1 pancreatic cancer cells after individual knockdown of Sp1, Sp3 and Sp4 demonstrates that these TFs regulate gene sets/pathways that both correlate and inversely correlate with the functional responses observed after knockdown. However, causal IPA analysis which integrates pathway-dependent changes in all genes strongly predicted that Sp1-, Sp3- and Sp4-regulated genes were associated with the pro-oncogenic activity. These functional and genomic results coupled with overexpression of Sp transcription factors in tumor vs. non-tumor tissues and decreased Sp1 expression with age indicate that Sp1, Sp3 and Sp4 are non-oncogene addiction genes and are attractive drug targets for individual and combined cancer chemotherapies.

2067 Chemically Induced Mammary Tumor Differences Between Rapid and Slow Arylamine N-Acetyltransferase Congenic Fischer 344 Rats Administered 7,12-Dimethylbenzanthracene (DMBA)

M. W. Stepp[4], M. A. Doll[4], D. J. Samuelson[4], M. A. G. Sanders[2], J. C. States[4] and D. W. Hein[4]. *[1]Department of Biochemistry & Molecular Genetics, University of Louisville, Louisville, KY; [2]Department of Pathology, University of Louisville Hospital, Louisville, KY; [3]James Graham Brown Cancer Center, University of Louisville, Louisville, KY and [4]Pharmacology & Toxicology, University of Louisville, Louisville, KY.*

Recent investigations suggest possible endogenous role(s) of arylamine N-acetyltransferase 1 (NAT1) in cancer progression. We conducted *in vivo* studies using F344 rats, congenic at the Nat2 locus for high (rapid) and low (slow) NAT2 activity. Rat NAT2 is a functional ortholog to human NAT1. Previously methylnitrosourea, a direct-acting alkylating agent not metabolized by NAT, induced mammary tumors in rats dosed at either age 3 or 8 weeks. In that study, congenic rapid acetylator rats exhibited higher susceptibility to tumors than did congenic slow acetylator rats. To confirm these findings with another carcinogen not metabolized by NAT, rapid and slow acetylator NAT2 congenic rats were dosed with 7,12-dimethylbenzanthracene (DMBA) at age 8 weeks. Over time congenic rapid acetylator rats again developed more palpable mammary tumors than slow acetylator rats, however log-rank test of tumor latency on a Kaplan-Meier plot found the difference non-significant (p=0.1013). At 23 weeks post-dosing, tumors were found in 76% of the rapid compared to only 43% of slow acetylator congenic rats. Both tumor incidence (p=0.0271) and multiplicity (p=0.0040) differed significantly between congenic rapid and slow acetylator rats. No apparent difference in tumor histopathology was observed between rapid and slow NAT2 congenic rats. Recombinant rapid rat NAT2 catalyzed folate-dependent acetyl-coenzyme A hydrolysis at higher rates than slow rat NAT2 and this result was also observed in tissue lysates derived from rapid and slow NAT2 congenic rats. The results further suggest an endogenous role for human NAT1 in tumorigenesis independent of its role in carcinogen metabolism that may involve regulation of acetyl coenzyme A levels.

2068 Prochloraz: Further *In Vitro* Data Comparing Responses in Male Wild-Type Mouse and Human Cultured Hepatocytes Completes the Human Relevance Framework

C. Strupp[1], S. Melching-Kollmuss[3], L. Chatham[2], D. Lowes[2], I. Fegert[3] and C. Elcombe[2]. *[1]Adama Europe, Schaffhausen, Switzerland; [2]CXR Biosciences Ltd., Dundee, United Kingdom and [3]Regulatory Toxicology Crop Protection, BASF SE, Ludwigshafen, Germany.*

The fungicide Prochloraz induced the formation of hepatocellular tumors after chronic administration to CD1 mice, but not SD or F344 rats. Mechanistic studies identified a non-genotoxic mode of action, with clear thresholds for induction of tumors. Prochloraz has been previously assessed *in vivo* in wild-type mice, constitutive androstane receptor (CAR)/pregnane X receptor (PXR) knock-out (KO) mice and humanised CAR/PXR mice and showed that mouse liver tumours were induced via a CAR-mediated Mode of Action which is considered not human relevant. In order to confirm non-human relevance, the response in isolated mouse hepatocytes in culture was compared to the response in cultured human hepatocytes. For Prochloraz, a similar response pattern was observed in cultured mouse hepatocytes as was determined in the short-term *in vivo* mouse study. Prochloraz increased the hepatocellular labelling index (S-phase) in a dose-dependent manner following exposure at different concentrations. Subsequently human hepatocytes were assessed for hepatocellular proliferation following Prochloraz exposure. There was no hepatocellular proliferation observed; the validity of the test system was proven by the use of positive standard (epidermal growth factor) as a proliferative stimulus. These data collectively demonstrate that Prochloraz can activate CAR and possibly PXR in both mouse and human hepatocytes. While proliferation was shown in mouse hepatocytes, no proliferation was measured in human hepatocytes. As CAR activation and cell proliferation are two key events in sustained liver activation-induced rodent liver tumours (Elcombe et al., 2014 Crit. Rev. Toxicol. 44: 64-82), the absence of cell proliferation in the human hepatocytes suggests that the increase in liver tumour formation mediated by Prochloraz in mice is not relevant to humans.

2069 Transgenerational Effects of Developmental Exposure to Low-Dose Zeranol on Sexual Development, Reproduction and Mammary Carcinogenesis

C. A. Lewis, B. Estrella, A. Green, J. Barrett, M. Gallo, J. Richardson and H. Zarbl. *Rutgers, Piscataway, NJ.*

Zeranol (Zer) is a potent semi-synthetic derivative of zearalenone, a mycoestrogen produced by Fusarium species that contaminate grain. Zer is deliberately introduced into livestock in the US as a growth promoter and developed as a substitute for the carcinogen diethylstilbestrol (DES). Zer is banned in Europe and Asia. Zer is detected in finished food products, is extremely stable at cooking temperatures and with a 22 hour half-life in humans. Occupational exposures to Zer are associated with precocious puberty and gynecomastia. Recent studies in prepubescent girls indicate that human exposure is primarily via the consumption of beef and corn, and urinary levels of unconjugated Zer are associated with altered onset of puberty, height and weight. Our studies in F344 rats indicated that developmental exposure to Zer (between GD7 to weaning) via the diet at doses below the human ADI (1.25µg/kg/day) resulted in precocious puberty (defined as a 3 day decrease in age at vaginal opening) in F1 progeny, increased uterine weight and abnormally prolonged estrous cycle. F1 male progeny showed feminization as assessed by decreased anogenital distance (AGD) and decreased sperm count. F1 females treated with a single carcinogenic dose of N-nitroso-N-methylurea (NMU) showed decreased latency, increased incidence of mammary tumors, increased tumor multiplicity and greater tumor mass. Similar effects on puberty and carcinogenesis were also observed in the F2 progeny, but only if both the dam and the sire were exposed to Zer, suggesting recessive epigenetic inheritance. Studies on the F3 generation demonstrated a trend towards decreased litter sizes, a significant decrease in fecundity and number of male offspring. F3 female progeny had delayed vaginal opening with prolonged estrous cycle and F3 male progeny had decreased AGD and sperm count. Together these studies suggest that perinatal exposure to the Zer produces transgenerational effects on sexual maturation and susceptibility to chemical-induced carcinogenesis. Supported by a Grant from The New Jersey Commission on Cancer Research and pilot funding from the Rutgers- Environmental and Occupational Health Sciences Institute, and the NIEHS CEED at Rutgers, the State University of NJ.

2070 AKT Phosphorylation of RNF6 Is Required for RNF6 Mediated Ubiquitination and Transcriptional Regulation of the Androgen Receptor

H. Shimelis[2], D. Deshmukh[1], Y. Xi[1] and Y. Qiu[1]. [1]*Department of Pharmacology & Experimental Therapeutics and Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD and* [2]*Division of Experimental Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

The Androgen Receptor (AR) and the PI3K/AKT/mTOR pathway play an important role in the progression of castration resistant prostate cancer (CRPC). RNF6 is an ubiquitin E3 ligase and is overexpressed in human hormone-refractory prostate cancer tissues and required for prostate cancer cell growth under androgen-depleted conditions. Earlier, we had shown that RNF6 modulates AR transcriptional activity and specificity by ubiquitinating AR. Here, we show that AKT binds to RNF6 and induces phosphorylation of RNF6. This phosphorylation was found to be required for the association of RNF6 with AR. Moreover, this phosphorylation modulated the RNF6 mediated ubiquitination and transcriptional activity of AR. Loss of AKT-induced RNF6 phosphorylation significantly reduced AR activity. Our data suggest that there is crosstalk between the cell survival PI3K/AKT/mTOR pathway and the androgen AR via RNF6 and that this crosstalk might drive prostate cancer progression under androgen depleted conditions.

2071 Mutagenesis and Carcinogenesis of Chronic Exposure to Low-Dose DEHP and its Metabolite MEHP in Mammalian CHO Cells

P.-Y. Lin[2], Y.-J. Chang[2], C.-Y. Tseng[2] and M.-W. Chao[2]. [1]*Biomedical Engineering, Chung Yuan Christian University, Taoyuan, Taiwan and* [2]*Bioscience Technology, Chung Yuan Christian University, Taoyuan, Taiwan.* Sponsor: M.-W. Chao.

DEHP, Di-(2-ethylhexyl) phthalate, the most common plasticizer used in manufactures. It can be leached into a liquid that comes in contact with plastics, suggesting concerns about the health risk of human exposure. Several *in vivo* studies have shown that DEHP can be metabolized and converted to MEHP, mono-ethylhexyl phthalate. The metabolite can be absorbed through the villi and travel in the circulation system then causes series damages. Investigations demonstrated an association with elevated induction of rat hepatic cancer, testicular cancer, and developmental toxicity under chronic MEHP exposure. However, the mechanism of carcinogenicity induced by DEHP or its metabolite MEHP remains unclear. Thus, we used Chinese hamster ovary (CHO) gpt transgenic AS52 cell as model. Acute results show that the parental chemical, DEHP, as well as MEHP derivatives caused dose-dependent decreases in cell survival, but with very different potencies: MEHP has the high potency; and DEHP lower. However, Cm-H2DCF-DA fluorescence and comet methods indicated a relative low level intracellular ROS and sequential DNA damages were generated after exposure to low dose of MEHP for 15 days. In 6-thioguanine-selection assay, the data show that the chronic exposure to 0.05 mM MEHP causes 2.5-fold increase of mutation fraction. Then the mutant clones (ASMC) were collected and analyzed with mRNA array. We found several oncogenes containing k-Ras, BRCA-2, FGFR, Smad-2, Akt3, JNK, Ski, Met, and c-Myc and Aryl hydrocarbon receptor (AhR) were significantly up-regulated. It was noted that simultaneous treatment of AhR inhibitor shows no difference in reduction of these oncogenes expression. *In vivo*, xenograft studies were performed with injecting 2.5X106/site ASMC cells subcutaneously per Nu-mouse. Obviously, 3 of 15 mice had tumorigenicity within 4 weeks. Therefore, chronic low dose of MEHP treatment are not only cytotoxic, but also carcinogenic to the mammalian CHO cell.

2072 Overexpression of Canonical Histone H3 Promotes Malignant Cellular Transformation

T. L. Des Marais, H. Sun, T. Kluz, F. Wu, Y. Liu, X. Zhang, A. Munoz, Q. Y. Chen, J. Brocato, D. Chen, C. Jin and M. Costa. *Environmental Medicine, NYU, Tuxedo, NY.*

The human canonical replication-dependent histone mRNAs exhibit a 3' stem loop structure, which is highly conserved throughout evolution. The expression of the histone protein during S-phase of cell division is regulated by stem-loop binding protein (SLBP). This highly conserved mechanism allows for proper control of histone gene expression during DNA replication and chromatin packaging. Our previous studies show that exposure to arsenic (As), a carcinogenic metal, reduces levels of SLBP and increases poly-adenylated H3.1 mRNA, indicating that increased expression poly-adenylated H3.1 independent of cell-cycle state may lead to malignant cellular transformation. We have overexpressed poly-adenylated H3.1 as well as H3 variant, H3.3, which differs from canonical H3 protein by 4 amino acids, into immortalized human bronchial epithelial cells (BEAS-2B). Overexpression of poly-adenylated H3.1 dramatically increased colony formation in soft agar, suggesting that increased poly-adenylated H3.1 caused these cells to exhibit anchorage-independent growth properties. Notably, cells overexpressing H3.3 did not have increased colony formation. Malignant cells are known to become more dyscohesive, and potentially metastatic, due to reduced cellular shear stiffness, calculated using Young's modulus. Cells overexpressing poly-adenylated H3.1 had reduced stiffness potentially making them transplantable, and thus more invasive. In order to assess potential tumor formation, H3.1 and H3.3 overexpressing cells were sub-cutaneously injected into nude mice. Interestingly, cells overexpressing poly-adenylated H3.1 were able to form tumors, while H3.3 could not, further indicating a poly-adenylated H3.1-specific effect. We conclude that overexpression of poly-adenylated H3.1 independent of cell cycle stage had the potential to promote malignant cellular transformation, a driving force behind carcinogenesis.

2073 3D Matrix to Measure Nickel Carcinogenesis: A Focus on Nuclear Protein 1 (NUPR1)

B. A. Narayanan, T. S. Blau, N. K. Narayanan and M. Costa. *Environmental Medicine, New York University, Tuxedo, NY.*

Although the mechanism (s) of action of nickel carcinogenesis is partly based on its ability to generate reactive oxygen species (ROS), one possible mechanism could be prolonged exposure on a target tissue altering specific protein functions, which is typical for action through epigenetic mechanisms. In this study we determined whether exposure to nickel chloride (Nicl2) induce hypoxia and activate the stress related protein NUPR1 and causes pre-neoplastic cellular changes, transformation and extracellular matrix (ECM) degradation. Our preliminary findings showed that Ni exposure to bronchial epithelial cells BEAS-2B and human PBMC-monocytes exhibit only moderate reactive oxygen species (ROS) activity, however showed a small increase in the level of NUPR1 at 150uM after 24 hr. To determine whether Ni induced NUPR1 activity is independent of ROS, activity, further study was conducted using transformed keratinocytes (HaCat) with chronic exposure to Nicl2 for a longer duration (72h) at lower dose (50uM). Our findings reveal an elevated level of ROS (>4 fold) and a parallel increase in the NUPR1 (>5 fold) activity, suggesting a cell specific ROS dependent long term effect on NUPR1 with Ni exposure in keratinocytes. To investigate the underlying mechanism(s) the nickel in inducing cellular transformation, and the impact on the extracellular matrix (ECM), we used a hyaluronic acid (HA) based 3D hydrogel matrix that mimic the *in vivo* conditions of an ECM. We found that the mononuclear cells migrate in to 3D matrix and developed invasive migratory colonies within 7 days. These preliminary findings indicate the impact chronic low dose nickel exposure as a risk factor for human cancer by degrading the ECM.

2074 Histone Deacetylase Inhibitors Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Dependent Targeting of Specificity Protein Transcription Factors

E. Hedrick[4], L. Crose[3], C. Linardic[3] and S. Safe[1,4]. [1]*Institute of Biosciences and Technology, Texas A&M Health Sciences Center, Houston, TX;* [2]*Pediatrics, Duke University Medical Center, Durham, NC;* [3]*Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC and* [4]*Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.*

The two major types of RMS, namely embryonal (ERMS) and alveolar (ARMS) are treated with cytotoxic drugs and this is accompanied by drug resistance and toxic side effects later in life. Therefore, development of innovative chemotherapeutic strategies is imperative and a recent genomic analysis suggested the potential efficacy of reactive oxygen species (ROS)-inducing agents. Here we demonstrate the efficacy of potent histone deacetylase (HDAC) inhibitors, panobinostat and vorinostat, as agents that inhibit tumor growth *in vivo*, induce apoptosis, and inhibit invasion of RMS cell lines RD and Rh30. These effects are due to epigenetic repression of c-Myc which leads to downregulation of miRs 17, 20a, 27a, upregulation of ZBTB 4, 10, 34 and subsequent downregulation of Sp transcription factors. We also demonstrate that this mechanism occurs in an ROS-dependent mechanism and treatment with the antioxidant glutathione reversed the effects of the HDAC inhibitor substantially, while the histone acetylation was unchanged. These results elucidate a novel mechanism of antineoplastic activity for panobinostat and vorinostat that lies outside of their canonical HDAC inhibitory activity and present a potential new antitumorigenic approach to treat RMS.

2075 Chromium Induces H4k16 Hypoacetylation by Inhibiting MOF Through NUPR1 Expression

Q. Y. Chen, X. Zhang, T. Kluz, M. Costa and H. Sun. *Department of Environmental Medicine, New York University School of Medicine, New York, NY.*

Chromate is a well-established carcinogen linked to respiratory cancer and has been found at half of US toxic waste sites. In spite of considerable research efforts, the mechanism of Cr(VI)-induced carcinogenesis remains largely unknown. A growing body of evidence suggests that Cr is capable of inducing epigenetic changes, such as DNA methylation, histone modification, and microRNA. Acetylation of histone lysine residues neutralizes the positive charge from their side chains and decreases the affinity between histone tails and DNA, which subsequently leads to increased DNA accessibility and transcriptional activation in the gene promoter region. However, the effect of Cr(VI) on histone acetylation is still not clear. Here, we report that BEAS-2B cells exposed to 5 or 10 uM of Cr(VI) for 24 hours resulted in a striking and dose dependent decrease in H4K16ac. Co-treatment with Cr(VI) and Benzo(a)pyrene diolepoxide (BPDE) induced further decrease in H4K16ac. Cr (VI) transformed cells showed considerable decrease in H4K16ac compared to the control cells, which further indicates the correlation between Cr(VI) exposure and hypoacetylation of H4K16. H4K16 acetylation is known as an important determinant in higher-order chromatin structure and active gene expression. Male Absent on the First gene (MOF) is a H4K16 specific acetyltransferase. Our results indicate that Cr(VI) exposure significantly reduced MOF activity while its expression levels remained the same, thus suggesting that Cr(VI) may affect MOF activity rather than its expression. Moreover, Nuclear protein 1 (NUPR1), a MOF inhibitor, was increased in Cr(VI)-exposed cells. Depletion of NUPR1 in cells by small RNA interference rescued Cr(VI)- reduced H4K16ac. Together, our results indicate that Cr(VI) inhibits MOF activity through inducing NUPR1 expression which subsequently leads to H4K16 hypoacetylation. Since hypoacetylation of H4K16 is a known hallmark of human cancer, the results from our study presents strong evidence supporting the role of H4K16 hypoacetylation in chromium carcinogenesis.

2076 Activation of Aryl Hydrocarbon Receptor (AhR) by Indole-3-Carbinol (i3c) Decreases Tumor Susceptibility in an Inflammatory Model of Colorectal Tumorigenesis

C. J. Diaz-Diaz[2], S. M. Ronnekleiv-Kelly[2], M. Nukaya[2], P. Geiger[2], C. Bradfield[2] and G. Kennedy[2]. [1]*Department of Surgery, University of Wisconsin-Madison, Madison, WI and* [2]*McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, WI.*

Colorectal cancer (CRC) is the third leading cause of cancer-related mortality in the United States. It is known that patients with Inflammatory Bowel Disease (IBD), a disease characterized by chronic inflammation of the intestinal tract, have a high risk for CRC. Recent studies have revealed that the aryl hydrocarbon receptor (AHR), a transcriptional factor and a mediator for xenobiotics, plays an integral role in intestinal homeostasis by regulating intestinal innate immune cells and inflammation. Therefore, we hypothesize that AHR is involved in the development of inflammation-associated CRC. To investigate our hypothesis, we measured colon tumor multiplicity induced by colon tumor initiator azoxymethane (AOM) and colon tumor promoter dextran sodium sulfate (DSS) in Ahr null and WT mice. We found that Ahr null mice had nearly three-fold higher number of colon tumors compared to WT mice ($P<0.0001$). This result suggested that AHR plays a protective role in inflammation-associated colorectal tumorigenesis. Moreover, we examined if activation of AHR by Indole-3-carbinol (I3C) improves inflammation-associated colorectal tumorigenesis. Colorectal tumor development induced by AOM/DSS was decreased in 92% in wild-type mice treated with I3C. In contrast, the suppression was not observed in I3C treated Ahr null mice. Our result demonstrated that activation of AHR induced by I3C suppress the development of inflammation-associated CRC.

2077 PMA-Induced Differentiation of Human Myeloid Leukemia Cells Requires Inhibition of cdc2

A. Nakasone, D. Vasilyeva, S. Fiedler, V. Lauerre, P. Dolinsky, M. Gaspard, H. Silverstein, G. Packert and X. Hu. *Barry University, Miami, FL.* Sponsor: J. Rayner.

The critical role of cdc2 (CDK1) in G2-M transition of the cell cycle control in mammalian cells has been well documented. However, whether this pluripotent CDK regulates cell differentiation is unclear. Thus, the effect of phorbol 12-myristate 13-acetate (PMA) on cell differentiation and the expression of cdc2 in human myeloid leukemia cell line, TF-1a were investigated. When TF-1a cells were treated with 10^-5, 10^-6, and 10^-7 PMA for 48 and 72 h, they showed marked macrophage-like changes, evidenced by significant decrease in nucleus/cytoplasm ratio and increase in the expression of IL-1β. PMA treatment also caused time-dependent inhibition of cdc2 in both cytosol and nucleus of the cells, with maximal inhibition being observed by 48 and 72 hours, which paralleled with the cell differentiation course. In contrast, there was no significant cell differentiation and inhibition of cdc2 being observed in control human myeloid leukemia TF-1 and MV4-11 cells in response to PMA treatment. PMA treatment also rapidly introduced phosphorylation of MAPK kinas (MEK and p44/42 MAPK), which persisted for 24 h, after which MEK and ERK returned to base level, at which time the expression of cdc2 was still significantly downregulated, as compared with control cells treated with DMSO. Pretreatment of TF-1a cells with sense cdc2 partially inhibited PMA-induced IL-1β. Moreover, cells treated with PMA showed marked reduction in the level of cdc2-pRb complexes. Taken together, our data suggests that inhibition of cdc2 is required for late differentiation of TF-1a cells in response to PMA stimulation. Whether activation of MAPK pathway inhibits expression of cdc2 is currently under investigation. (Supported by Faculty Incentive Grant and NIH-NIGMS MBRS RISE: GM059244-15, Barry University)

2078 Penfluridol Suppresses Glioblastoma Tumor Growth by Inhibiting Sonic Hedgehog Signaling

A. Ranjan and S. Srivastava. *Biomedical Sciences, Texas Tech University Health Sciences Center, Amarillo, TX.*

Glioblastoma (GBM) is the most common brain tumor with poor survival rate. The main obstacle in the treatment of glioblastoma patients is the presence of blood brain barrier, which restricts the movement of the drugs to reach the brain. Penfluridol (PF) is known to cross blood brain barrier and is a clinically approved drug for schizophrenia patients. It is demonstrated and established by us that penfluridol suppresses the growth of metastasized breast cancer cells in brain giving us the rationale to evaluate it against glioblastoma (Alok Ranjan, Parul Gupta and Sanjay Srivastava, Cancer Research 2015). Penfluridol significantly reduced the viability of U87-MG, T98G and U251 MG glioblastoma cells with an IC50 of 6μM, 5.5μM and 9μM respectively after 24 h of treatment and induced apoptosis as exhibited by FITC/Annexin assay and cleavage of caspase 3 as well as PARP. It has been shown that GLI1, a transcription factor belonging to sonic hedgehog signaling is overexpressed in GBM and responsible for tumor progression. In addition, overexpression of MGMT, a DNA repair enzyme has been linked to temozolomide (TMZ) resistance in GBM therapy. Our results demonstrated that penfluridol treatment reduced the expression of GLI1 and MGMT in U87MG, T98G and U251 MG cells in concentration-dependent manner and sensitized GBM tumor cells to TMZ treatment. Combination of PF and TMZ induced more apoptosis and reduced the expression of GLI1 and MGMT as compared to PF or TMZ treatment alone. Our results further demonstrated that oral administration of PF inhibited the growth of GBM tumors by 66% and 85% in subcutaneous and intracranial GBM tumor model respectively. Immunohistochemical analysis of tumor tissue and western blot analysis of tumor lysate indicated down regulation of GLI1, MGMT and increase in cleavage of caspase 3, confirming *in vitro* finding. Taken together, these results indicate that overall GBM tumor growth suppression by penfluridol was associated with inhibition of Gli1 and MGMT and induction of apoptosis.

2079 Transplacental Dibenzo[def,p]Chrysene Exposure, Indole-3-Carbinol Intervention, and Alteration of Epigenetic Machinery

T. A. Harper[2], D. A. Sampson[2], J. Morre[2] and D. E. Williams[2]. [1]*Department of Chemistry, Oregon State University, Corvallis, OR and* [2]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Our laboratory previously reported that transplacental exposure to the polycyclic aromatic hydrocarbon (PAH) dibenzo[def,p]chrysene (DBC) results in an aggressive lymphoma and lung cancer in murine offspring. Additionally, maternal dietary intervention using indole-3-carbinol (I3C) increased the survival rate of DBC-exposed offspring and reduced the tumor multiplicity of lung cancer in surviving mice. Nonetheless, the mechanisms for DBC-induced transplacental carcinogenesis and I3C chemoprevention remain unknown. We hypothesize that I3C supplementation can protect the fetal epigenome, thereby preventing DBC-induced carcinogenesis after an *in utero* exposure. Pregnant dams were provided control AIN 93G diet or diet containing 500 ppm I3C on gestation day (GD) 9 until weaning. DBC or corn oil was administered via gavage on GD17. DBC-adduct formation in neonate lung was analyzed using UPLC-MS/MS. Additionally, epigenetic modulators were analyzed using qRTPCR, and western blot analysis. Our results show I3C did not reduce adduct formation in the lung of neonate mice. However, I3C attenuated a DBC-dependent increase in Hdac2 protein expression in neonate lung. Global DNA methylation in neonate lung paralleled Dnmt1 protein expression. As a result we are now in position to further investigate dietary modulation of the epigenome focusing on protein (de)acetylation mediated through Hdac2 and downstream consequences such as altered DNA methylation. Supported by NIH grants P01 CA90890, T32 ES007060, P42 ES016465.

2080 Diindolylmethane Analogs Are Ligands for NR4A Nuclear Receptors in Colon Cancer Cells

X. Li[1] and S. Safe[1,2]. [1]*Department of Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX and* [2]*Institute of Biosciences & Technology, Texas A&M University Health Science Center, Houston, TX.*

Members of the nuclear receptor subfamily NR4A include NR4A1 (TR3), NR4A2 (NURR1) and NR4A3 (NOR1) and they are overexpressed in many solid tumors. Knockdown studies by RNA interference indicate that these receptors play pro-oncogenic roles in several cancer cell lines. In this study, we have developed a group of structurally related 1,1-bis(3'-indolyl)-1-(p-substituted phenyl)methane (C-DIM) compounds as ligands to target these oncogenic NR4A receptors. Treatment with compounds such as p-hydroxyphenyl, p-cyanophenyl and p-carboxymethylphenyl C-DIM analogs significantly decreased transactivation of reporter gene in colon cancer cells transfected with NR4A response element reporter constructs. In addition, treatment with C-DIM compounds also inhibited cell proliferation and induced apoptosis in colon cancer cells. We have also cloned the ligand binding domains of NR4A receptors and purified each NR4A-LBD protein using affinity and size-exclusion chromatography methods. The ligand binding domains for these receptors include amino acids 355-598 (NR4A1), 353-598 (NR4A2) and 353-628 (NR4A3). Using fluorescence quenching assay and isothermal titration calorimetry, we have investigated the interaction between C-DIM ligands and NR4A-LBD proteins and the preliminary data indicated that these interactions were in the submicromolar to micromolar range.

2081 Global De-Regulation of Gene Expression in C3H/10T1/2 Cl 8 Mouse Embryo Cell Lines Transformed by Insoluble Ni(II) Compounds

J. R. Landolph[2], A. T. Pehl-DeSilva[2] and K. A. Akinwumi[2]. [1]*Dept. of Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA and* [2]*Dept. of Pathology, University of Southern California, Los Angeles, CA.*

Nickel (Ni) refinery workers inhaling Ni-containing sulfidic ore dusts and smoking cigarettes in Ni refineries contracted lung/nasal cancers. Inhalation of Ni3S2 or green NiO induces respiratory cancer in rats. Ni3S2 and green/black NiOs were phagocytosed into/induced chromosomal aberrations, cytotoxicity, and morphological, A. I., and neoplastic transformation in, 10T1/2 mouse embryo cells. 144 genes were differentially expressed between non-transformed and 2 MCA/4 NiO or NiS-transformed (Tx) 10T1/2 cell lines (mRNA differential display). Ni-/MCA-Tx cell lines had a)ect-2 gene amplification/higher levels of ect-2 gene mRNA/protein; b) higher levels of calnexin mRNA/protein and Wdr1 mRNA; c) no detectable DRIP80 or β-centaurin-2 mRNAs. We hypothesized 1) Ni+2-induced amplification of ect-2 gene led to higher levels of microtubules (MTs); 2) Ni+2-induced silencing of β-centaurin-2 gene caused higher levels of microfilaments (MFs); and 3) Ni+2-induced silencing of DRIP80 gene altered Ca+2 ion distributions, in Tx 10T1/2 cells. To test these hypotheses, we stained cells with: fluorescent phalloidin to decorate MFs; fluorescent Ab to α-tubulin to decorate MTs; and DAPI to decorate nuclei, then examined cells by confocal microscopy. In non-Tx 10T1/2 cells, MFs/MTs were arranged homogeneously in long fibers. In NiS/green NiO-Tx cell lines, MFs/MTs were over-expressed in some areas, absent in others, changing shapes of Tx cells. In 6 NiO/NiS- or MCA-Tx cell lines, Ca+2 ions were largely cytoplasmic. Our data suggests mutations in 6 target genes and silencing 9 other target genes led to differential expression of 10 genes for each primary gene altered, causing differential expression of 144 genes in Tx cell lines. We conclude: 1) Ni+2 ions amplified ect-2, leading to expression of higher steady-state levels of MTs, and silenced β-centaurin-2, causing expression of higher steady levels of MFs, and changes in cell shape and gene expression in Tx cell lines. 2) Ni+2 ions silenced DRIP80 gene, altering Ca+2 distributions in Tx 10T1/2 cell lines. 3) These Ni ion-induced events caused differential expression of 144 genes, and induction/maintenance of Tx phenotypes in Ni+2/ MCA-Tx cell lines.

2082 Pentoxifylline Modulates Major Growth and Metabolic Pathways in Prostate Cancer Cells

R. D. Canatsey[1,2], G. T. Le[1,2], T. J. Monks[1,2] and S. S. Lau[1,2]. [1]*Dept. of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ and* [2]*Southwest Environmental Health Sciences Center, Tucson, AZ.*

Environmental exposure to carcinogens modulates growth-signaling pathways and causes oncogene activation in many cancers. Targeting these growth pathways has proven to be an effective therapeutic strategy in multiple cancers; however metastatic prostate cancer prognosis remains poor. Cyclin D1 is required for progression from the G1 phase into the S phase of the cell cycle. Over-expression of cyclin D1 causes a concomitant increase in cell cycle progression and cell proliferation, suggesting it plays a role in tumor formation. Mechanistic studies designed to understand the modulation of cyclin D1 expression as a potential anticancer target revealed that pentoxifylline (PT, 3.6 mM), a clinically utilized phosphodiesterase inhibitor, decreased cyclin D1 protein levels in human prostate adenocarcinoma (LNCaP) cells. PT exhibited a dose and time dependent inhibition of LNCaP cell growth assessed by MTS viability assay, cell cycle analysis, and light microscopy. PT also induced a transient decrease in the activity of the AKT and AMPK pathways, with a sustained downregulation of the downstream proteins phospho-4EBP1 and cyclin D1. Moreover, PT induced the cleavage of LC3, which may be indicative of autophagy engagement. In combination with docetaxel, the first line therapy for prostate cancer, PT effectively decreased (>10 fold) the dose of docetaxel required for an appreciable loss of cell viability. Combination dosing of PT and docetaxel (3.6mM and 10nM) inhibited PT induced LC3 cleavage, but concomitantly produced a more rapid and prominent induction of apoptotic markers, including caspase-3, -7 and PARP cleavage, and an increase in annexin staining. Interestingly, PT effectively inhibited docetaxel induced p53 induction, suggesting an additional potential benefit for combination therapy. In summary, our findings demonstrate a novel anti-proliferative property of PT, which may serve as an effective adjunct therapy in prostate cancer. (P30ES006694, T32ES007091).

2083 The Role of PPARγ During Breast Tumour Angiogenesis

J. Y. Shi[2], A. J. Apostoli[2], R. E. Rubino[2] and C. J. Nicol[2]. [1]*Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada and* [2]*Division of Cancer Biology and Genetics, Cancer Research Institute, Queen's University, Kingston, ON, Canada;* [3]*Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada.*

Breast cancer-related deaths result from tumour growth and spread, to which angiogenesis is a key contributor. Previously, we showed for the first time that peroxisome proliferator-activated receptor (PPAR) γ signaling in mammary stromal endothelial cells (ECs) suppresses 7, 12-dimethylbenz[a]anthracene (DMBA)-mediated breast tumourigenesis *in vivo*. PPARγ activating drugs are reported to have anti-angiogenic effects, but the role of PPARγ signaling during breast tumour angiogenesis is unknown. Here, it was hypothesized that EC loss of PPARγ would alter angiogenic signaling during breast tumour angiogenesis. Serum was isolated from whole blood collected from (n=5/group) untreated 8-12 week old wildtype (WT) and EC-targeted PPARγ-knockout (PPARγ-EC KO) mice, or (n=4/group) mice at necropsy post treatment with DMBA (1mg/week p.o.) for 6 weeks and either continued on nor-

mal chow diet at week 7 (DMBA Only) or a diet supplemented with a PPARγ ligand (rosiglitazone, 4mg/kg/day) (DMBA+ROSI) for 25 weeks. Serum was assessed using a Mouse Cytokine 23-plex serum assay kit for expression differences. PPARγ-EC KO serum expression of several interleukins (5, 6, 10, and 17α), chemotaxins (eotaxin, CXCL1, MCP-1, and MIP-1β), and inflammatory factors (G-CSF, GM-CSF, and TNFα) were significantly lower than those in WT mice ($p<0.05$). In contrast, DMBA significantly decreased IL-5 and increased IL-10 and CXCL1 in PPARγ-EC KO but not WT mice ($p<0.05$). DMBA+ROSI significantly increased IL-1α and decreased IL-10 in PPARγ-EC KO but not WT mice ($p<0.05$). Further, studies using untreated aortae isolated from PPARγ-EC KO mice showed a trend toward higher EC sprouting versus WTs upon angiogenic (VEGF) stimulation. Together, these data are the first evidence that loss of EC-specific PPARγ alters the angiogenic environment during breast tumourigenesis, and support an early anti-angiogenic role for activating PPARγ signaling in breast cancer patients.

2084 microRna Expression Changes in the Liver of Fisher 344 Rats After Furan Exposure

A. de Conti, V. Tryndyak, F. A. Beland, D. R. Doerge and I. Pogribny. *Biochemical Toxicology, NCTR, Jefferson, AR.*

Furan is a heterocyclic organic compound produced in the chemical manufacturing industry and is also found in a broad range of food products, including infant formulas and baby foods. Previously, we demonstrated that the adverse biological effects of furan, including its liver tumorigenicity, may be associated with epigenetic abnormalities; however, microRNA (miRNA)-response to furan exposure remained unexplored. In the present study, we investigated the expression of several selected cancer-related miRNAs in the livers of Fisher 344 (F344) rats treated with different doses of furan (0.92, 2.0, 4.4 and 8 mg/kg/day body weights) during exposure and after cessation of exposure. The results demonstrate that all furan doses resulted in early and persistent over-expression of hepatic miRNAs, miR-34a, miR-93, miR-194, miR-200a, miR-200b, and miR-224, and down-regulation of miR-375. Interestingly, a majority of furan-induced miRNA changes, except miR-375, were transient and became restored after cessation of the furan treatment. In contrast, the expression of miR-375, which is one of the most significantly down regulated miRNAs in liver cancer, steadily decreased in a time-dependent manner following furan treatment. In summary, the results of the present study indicate that furan-induced adverse effects may be mechanistically related to a sustained decrease in the expression of tumor suppressor miRNA miR-375, which, in turn, caused aberration in key cancer-related pathways, including cell proliferation, apoptosis, autophagy, and cell migration. Moreover, this study provided evidence on the importance of miRNA alterations in cancer development and emphasized the potential for miRNA changes as early indicators of the carcinogenic process.

2085 Linking Site-Specific Loss of Histone Acetylation to Repression of Gene Expression by the Mycotoxin Ochratoxin A

E. Limbeck[2], J. T. Vanselow[2], J. Hofmann[2], A. Schlosser[2] and A. Mally[2]. [1]*Department of Toxicology, University of Wuerzburg, Wuerzburg, Germany and* [2]*Rudolf Virchow Center, University of Wuerzburg, Wuerzburg, Germany.*

Ochratoxin A (OTA) is one of the most potent renal carcinogens but its mechanism has not been fully resolved. Gene expression profiling studies revealed down-regulation of gene expression as the predominant transcriptional response to OTA *in vitro*. Recently, we showed that OTA directly inhibits histone acetyltransferases (HATs), thereby causing a dose-dependent reduction of lysine acetylation of histones and non-histone proteins. Considering the role of specific histone acetylation marks in the regulation of gene transcription, we speculated that OTA-mediated repression of gene expression may be linked to inhibition of HAT activity and subsequent histone hypoacetylation. In this study, we used a novel mass spectrometry approach for quantification of posttranslational acetylation sites based on chemical acetylation of unmodified lysine residues using 13C-labeled acetic anhydride, to analyze site-specific alterations in histone acetylation in HK-2 cells exposed to OTA. Our results demonstrated OTA-mediated loss of acetylation at almost all lysine residues of histones H2A, H2B, H3 and H4. Since acetylation at histone H3 lysine 9 (H3K9), which was reduced from ~ 3% in controls to < 0.1% in response to OTA, is well-known to be associated with an open chromatin structure that allows access of transcription factors to promoter regions of active genes, we further used chromatin immunoprecipitation followed by quantitative real-time PCR (ChIP-qPCR) to study a possible link between H3K9 hypoacetylation and OTA-mediated down-regulation of selected genes (AMIGO2, CLASP2, CTNND1). ChIP-qPCR analyses revealed OTA-mediated loss of H3K9 acetylation at the promoter regions of these genes (% of untreated controls: AMIGO2: 45%, CLASP2: 60%, CTNND1: 54%). These data provide first evidence that H3K9 hypoacetylation as a consequence of OTA-mediated inhibition of HATs may be a primary mechanism by which OTA modulates gene expression.

2086 Formaldehyde-Induced DNA Damage in Bone Marrow

R. Yu[3], B. Moeller[1], M. Doyle-Eisele[1], D. Kracko[1], W. Bodnar[3], L. Pontel[4], K. Patel[4], T. Starr[2,3] and J. Swenberg[3]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM;* [2]*TBS Associates, Raleigh, NC;* [3]*Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC and* [4]*MRC Laboratory of Molecular Biology, University of Cambridge, Cambridge, United Kingdom.*

Rationale: Epidemiologic associations have been reported between formaldehyde exposure and the induction of leukemia. However, whether or not inhaled formaldehyde exposure causes leukemia remains debatable. Previous experimental results have not supported the induction of leukemia, and epidemiological reports have been inconsistent across different studies. The purpose of this study was to determine the amounts of DNA damage in bone marrows in various animals and using different methods for collection. Methodology: DNA from bone marrow (mouse, rat, monkey, and human) were isolated, reduced, digested, and injected onto an Agilent HPLC-UV for adduct fractionation. DNA mono-adducts were quantitated using nanoUPLC-MS-MS (with a limit of detection of 0.5 attomoles). Results: Endogenous, but not exogenous DNA damage was observed in all bone marrows. The amount of endogenous DNA damage in monkeys was found to be extremely high in scraped bone marrow compared with traditional saline extrusion. We are currently analyzing human scraped bone marrow. Conclusions: Because the saline extruded bone marrow is likely to contain predominantly blood, the number of DNA adducts in these samples would be diluted by the relatively low amount of DNA adducts present in blood. In contrast, the large number of DNA adducts found in direct bone marrow scraping reflects the true value of endogenous DNA damage. These data support our hypothesis that endogenous formaldehyde induced DNA damage in bone marrow could result in "spontaneous" mutations and thus may represent an important source of leukemia and bone marrow failure.

2087 The Relationship Between Forestomach Hyperplasia and Tumorigenesis in Rodents Following Chronic Oral Exposure to Possible Carcinogens

J. M. Fritz[1], M. R. Gwinn[1] and C. E. Wood[2]. [1]*National Center for Environmental Assessment, US EPA, Washington, DC and* [2]*National Health and Environmental Effects Research Laboratory, US EPA, Research Triangle Park, NC.*

Hyperplasia is an increase in cell number that often arises as a response to chemical-induced cytotoxicity. In mode of action evaluation, this finding may also serve as an important intermediate event in the carcinogenic process. Squamous hyperplasia and neoplasia are frequently detected in the rodent forestomach (FS) following chronic oral administration of chemicals. In order to further inform the use of rodent FS lesions in human health hazard assessment, the relationship between FS hyperplasia and tumor induction was evaluated. Genotoxicity test results and FS lesion classification and incidence data were obtained from rat and mouse oral (primarily gavage) bioassays on the 78 chemicals from National Toxicology Program (NTP) 2-year bioassays which reported dose-related increases in FS hyperplasia or tumors in at least one rodent sex/strain combination, representing approximately 13% all NTP bioassays. The relationship between FS hyperplasia and tumor (e.g., squamous cell papilloma or carcinoma) induction was investigated in this database, and compared against information from the EPA ToxRefDB (primarily feed) bioassays. In mice, equal numbers (20%) of NTP agents were associated with either FS hyperplasia or tumor induction, or both. Conversely, <8% of NTP test compounds caused only FS neoplasia in rats, while ~30% were associated with only FS hyperplasia. In NTP compounds characterized as genotoxic, FS tumor and hyperplasia incidence was positively associated in rats; this relationship was not apparent with non-genotoxic compounds in rats, or with either group in mice. This analysis may inform tumorigenicity predictions when only information from shorter-term animal or *in vitro* studies is available, as well as guide mode of action interpretation in more extensive databases. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

2088 Transcriptional Profiling of Rats Exposed to Acrylamide Does Not Support a Genotoxic Mode of Action for Thyroid Cancer

N. Chepelev[2], R. Gagné[1], L. Recio[2] and C. Yauk[1]. [1]*Health Canada, Ottawa, ON, Canada and* [2]*Integrated Laboratory Systems, Research Triangle Park, NC.*

Acrylamide exposure leads to thyroid, but not liver tumors in F344 male rats exposed to 2.0 mg/kg body weight per day (bw-day) for two years. The mechanisms leading to this tissue-specific carcinogenicity are unclear. We used RNA-sequencing to explore the pathway perturbations contributing to acrylamide-induced thyroid cancer. Male F344/DuCrl rats were given 0, 0.5, 1.5, 3.0, 6.0, or 12.0 mg acrylamide/kg bw-day in drinking water for 5, 15, or 31 days. This design uses the same route of administration and spans and exceeds the doses used in the cancer bioassays. Thyroid glands and livers were harvested and mRNA was sequenced (20 million reads per sample) by poly-A RNA-sequencing on an Ion Proton sequencer. Ingenuity Pathway Analysis was used to explore the molecular perturbations induced by acrylamide. There were 261, 485, and 164 genes differentially expressed (p-value < 0.001, fold-change > 1.5) in the thyroid glands after 5, 15, or 31 days of exposure, respectively. The canonical pathway "Calcium Signaling" was significantly affected 5- and 15-, but not 31-days post-exposure. At 31 days, cell function/maintenance and signalling, and inflammatory/immunologic processes were perturbed. In the liver, there were remarkably few changes in gene expression. The largest response was 137 differentially expressed genes (p-value < 0.001, fold-change > 1.5) for the highest dose on day 31, with significant perturbations in genes involved in chromosomal segregation (the most significantly enriched gene ontology term). In addition, the results of micronucleus assay and the Pig-a mutation assay were negative at all doses (N-ethyl-N-nitrosourea was used as positive control). This study supports earlier reports of the effects of acrylamide on cytoskeletal proteins, suggesting that acrylamide causes rat thyroid tumors by impairing chromosome segregation during cell division through transcriptional effects on calcium/cytoskeleton proteins, but not by direct DNA damage.

2089 Delayed Scopolamine Efficacy Following Nerve Agent Challenge in the Hartley Guinea Pig

J. L. Plahovinsak, M. Edwards, N. A. Niemuth and J. A. Harvilchuck. *Battelle, Columbus, OH.*

A combined regimen of pretreatment and therapy is believed to be the most promising use of countermeasures to protect the warfighter against a nerve agent attack. The current treatment regimen includes the rapid administration of an anticholinergic drug (atropine), an oxime reactivator (pyridine-2-aldoxime methylchloride; 2-PAM Cl) and an anticonvulsant. This study evaluated the efficacy of a delayed administration of scopolamine (SCP) when compared to administration at 1 min post-challenge in the Hartley guinea pig after challenge with nerve agent. This study also determined the efficacy of SCP when used as an adjunct to the current treatment regimen, with or without pyridostigmine bromide (PB) pretreatment. Primary endpoints were 8 and 24 hr survival and clinical observations to include quality of life (QOL). Animals were pretreated with 0.026 mg/kg PB or saline 30 min prior to challenge with either sarin (GB) or soman (GD). One min post-challenge animals were administered atropine (0.4 mg/kg), 2-PAM Cl (25.7 mg/kg) and midazolam (0.66 mg/kg). At 1, 30, 60 or 120 min post-challenge, surviving animals were administered SCP (0.1 mg/kg; IM). QOL scores were determined from clinical observations were taken prior to PB, prior to challenge, and through 24 hours post-challenge. For GB and GD challenge (with or without PB), the delay of SCP through 120 min post-challenge did not significantly decrease efficacy when compared to rapid SCP administration at 1 min post-challenge. The delayed administration of scopolamine resulted in a decreasing trend in both 24 hours survival and QOL scores; however, a larger number of animals would be required to determine whether that trend was statistically significant. These findings conclude that the early administration, optimally at 1 min post-challenge, of SCP following a nerve agent challenge increases survival and QOL in the guinea pig. This work was supported by contract number SP0700-00-D-3180. This work was developed in collaboration with Medical Countermeasures Systems, US Army Medical Research Institute for Chemical Defense and the Defense Threat Reduction Agency. The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. Distribution Statement A: Approved for public release;
distribution is unlimited.

2090 *In Vitro* Inhibition/Reactivation of Rat Serum Butyrylcholinesterase After Exposure to Nerve Agent Surrogates and OP Insecticidal Oxons

R. Nichols[2], H. Chambers[2] and J. Chambers[2]. [1]*Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Starkville, MS and* [2]*Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Starkville, MS.*

Organophosphates (OP), such as nerve agents and insecticides, are potent inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Oxime reactivator treatment is a critical therapeutic in OP poisoning. However, the current reactivation therapeutic (2-PAM) is not broad spectrum, and is not an effective BChE reactivator. BChE inhibition does not lead to toxicity, which suggests a role for BChE as a potential bio-scavenger. BChE inhibition by the OP followed by reactivation in the serum could provide a pseudo-catalytic mechanism and potentially prevent severe signs of toxicity. Having an oxime that can reactivate BChE could provide additional antidotal therapy in OP poisoning. Our laboratory has synthesized a series of phenoxyalkyl pyridinium oximes. This study examined the reactivation potential of these novel oximes (100μM) on BChE in rat serum after exposure to phthalimidyl isopropyl methylphosphonate (PIMP; a sarin surrogate), nitrophenyl ethyl methylphosphonate (NEMP; a VX surrogate), nitrophenyl cyclohexyl methyl phosphonate (NCMP; a cyclosarin surrogate), diisopropyl fluorophosphate (DFP; a commonly used surrogate for nerve agent testing), and two insecticidal OPs: paraoxon (PXN; metabolite of parathion) and phorate oxon (PHO; metabolite of phorate). Tested OP concentrations yielded about 80% inhibition. Reactivation results indicated a range of 45-68% against PIMP, 18-62% against NEMP, 0-21% against NCMP, 0-12% against DFP, 33-70% against PXN, 0-24% against PHO. 2-PAM (100μM) reactivation was 46% against PIMP, 8% against NEMP, 4% against NCMP, 2% against DFP, 32% against PXN, and 70% against PHO. These *in vitro* data suggest that several of these novel oximes are substantially better reactivators of BChE than 2-PAM against PIMP, NEMP and PXN, showing promise as an additional therapeutic. (Partially supported by NIH U01 NS083430)

2091 Novel Substituted Phenoxyalkyl Pyridinium Oximes Enhance Survival of Rats Receiving Lethal Levels of Nerve Agent Surrogates and Paraoxon

R. B. Pringle, E. C. Meek, H. W. Chambers, C. A. Leach and J. E. Chambers. *College of Veterinary Medicince, Mississippi State University, Mississippi State, MS; Dept. Biochem., Mol. Bio., Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS.*

Novel substituted phenoxyalkyl pyridinium oximes, previously shown to reactivate brain acetylcholinesterase in rats treated with high sublethal dosages of surrogates for sarin and VX were tested for lethality prevention from lethal doses of these organophosphates. Rats were treated subcutaneously with 0.6 mg/kg nitrophenyl isopropyl methylphosphonate (NIMP; sarin surrogate), 0.65 mg/kg nitrophenyl ethyl methylphosphonate (NEMP; VX surrogate) or 0.8 mg/kg paraoxon (PXN). Animals receiving these dosages died within 24 hours when only 0.65 mg/kg atropine (human equivalent dosage) was administered at the onset of seizure-like behavior (around 30 min). When 146 mmol/kg 2-PAM was also given, 24 hour survival was 40, 33, and 50% for NIMP, NEMP, and PXN, respectively. Novel oximes 1 and 20 provided 65 and 55% survival for NIMP, 75 and 65% for NEMP, and 35 and 30% for PXN, respectively. These oximes also significantly reduced the cessation time of seizure-like behavior compared to 2-PAM during the first 8 hours of observation. Inhibition of brain acetylcholinesterase in 24 hour survivors was also slightly reduced in novel oxime treated rats compared to 2-PAM. The lethality data indicate that these two novel oximes enhance 24 hour survival compared to 2-PAM for NIMP and NEMP (but not PXN). Additionally, the earlier cessation of seizure-like behavior strongly suggests that these novel oximes penetrate the blood-brain barrier and alleviate the excessive cholinergic activity that results in seizures. Ultimately, these oximes have notable therapeutic potential as antidotes for nerve agents via prevention of nerve agent-induced lethality and early attenuation of subsequent seizures. (Supported by NIH U01 NS083430).

2092 Analysis of Rat Brain Acetylcholinesterase (AChE) Reactivation by Novel Oximes and Pralidoxime Chloride (2-PAM) After Inhibition with a Cyclosarin Surrogate

S. A. Dezell[2], H. W. Chambers[2] and J. E. Chambers[2]. [1]*College of Veterinary Medicine, Mississippi State University, Mississippi State, MS and* [2]*Dept. Biochem., Mol. Bio., Entomolgy and Plant Pathology, Mississippi State University, Mississippi State, MS.*

The use of nerve agents for military strategy and terrorism continues to be a problem as seen recently with the sarin gas used in the Syrian civil war. Treatment involves atropine to block the effects of the nerve agent and 2-PAM to reactivate inhibited AChE. 2-PAM is not an effective reactivator of AChE inhibited by sarin or cyclosarin. Our laboratories have invented a series of novel substituted phenoxyalkyl pyridinium oximes (patent pending) which are effective reactivators of AChE inhibited by a sarin surrogate, nitrophenyl isopropyl methylphosphonate (NIMP). The objective is to characterize the *in vitro* rat brain AChE reactivation activity of these novel oximes after inhibition with the cyclosarin surrogate 4-nitrophenyl cyclohexyl methylphosphonate (NCMP) or 2-PAM. A modified Ellman's AChE activity assay was used to compare our oximes to 2-PAM. Three out of 17 total tested oximes were able to reactivate inhibited AChE at levels similar to 2-PAM at 5.7% when oxime was used following the inhibition incubation. Focusing on the 3 successful oximes, simultaneous incubation of the novel oximes and NCMP improved reactivation to 34.4% versus 2-PAM at 5.1%. In addition, combined studies with 2-PAM and the novel oximes increased reactivation to 39.5%. While a higher reactivation percentage is desired, the results indicate oxime structures that could be further modified to increase their effectiveness against NCMP inhibition. We have also demonstrated possible use of combinations of various oximes that may result in an effective antidote for sarin or cyclosarin. (Support in part from NIH U01 NS083430).

2093 Determination of the Median Effective Dose of Oxime Therapies Against Topical Challenges of Organophosphates in the Guinea Pig

M. C. Babin[1], T. H. Snider[1], D. A. Jett[3], G. E. Platof[2] and D. T. Yeung[3]. [1]*Battelle, Columbus, OH;* [2]*National Institutes of Health National Institute of Allergy and Infectious Diseases, Bethesda, MD and* [3]*National Institutes of Health National Institute of Neurological Disorders and Stroke, Bethesda, MD.*

The antidote against organophosphate (OP) is atropine with an oxime, and diazepam. Previously, we determined (1) the relative efficacy of eight oximes against the 85th percentile lethality topical dose of four OP challenges and (2) the protective ratio of four select oximes used with atropine against topical challenges of the same OPs. The objective of this follow-on study was to determine the median effective dose (ED50) of select oximes against topical challenges at 2x median lethal dose (LD50) of three OPs. Male guinea pigs received neat applications of phorate oxon (PHO, 196 mg/kg), VX (0.240 mg/kg), or Russian VX (VR, 0.365 mg/kg) and, beginning with toxicosis, received therapy by IM injection of atropine free base (0.4 mg/kg) followed by an oxime at varying levels. Clinical signs, recorded at 3 hr post-therapy, were plotted versus log(oxime treatment dose) by OP. Probit analysis was used to calculate the 3hr ED50 with 95% confidence limits and slope. The therapeutic index (TI = oxime 24hr LD50/oxime ED50) was calculated for each OP/oxime. Also, the 3hr 90th percentile dose (ED90) for each oxime was contrasted with the molar equivalent of 2PAM Cl contained in three DuoDote® injectors for a 70kg person, 149 µmol/kg. The following data are for ED50 (µmol/kg) and TI values. Against a PHO challenge, obidoxime Cl2 (2.3; 35) and HLö-7 DMS (5.5; 57) performed better than MMB4 DMS (14; 50), and 2-PAM Cl (33; 5.4). Against VX, the order of effective oximes was MMB4 DMS (11; 64) > HLö-7 DMS (27; 12) > obidoxime Cl2 (40; 2.0) > 2-PAM Cl (81; 2.2). Against VR, the order of effective oximes was MMB4 DMS (17; 40) > HLö-7 DMS (72; 4.4), whereas 2PAM Cl and especially obidoxime Cl2 were regarded as ineffective. Overall, MMB4 DMS appeared to be the most effective oxime, because only MMB4 DMS exhibited ED90 < 149 µmol/kg against all three topical OPs.

2094 Nitrogen Mustard Upregulates Peroxisome Proliferator Activated Receptor (PPAR)-α in Mouse Skin

L. B. Joseph[2], G. M. Composto[2], I. M. Wohlman[2], H. D. Kim[1], D. R. Gerecke[2], D. L. Laskin[2], J. D. Laskin[2] and D. E. Heck[1]. [1]*New York Medical College, Valhalla, NY and* [2]*Rutgers University, Piscataway, NJ.*

Nitrogen mustard (bis(2-chloroethyl)methylamine, HN2) is a potent skin vesicant causing inflammation, edema and ulcerative wounds. In humans, exposure to HN2 can cause persistent tissue injury and blistering. The PPAR family consists of nuclear transcription factors that regulate cellular proliferation and differentiation as well as inflammation. Lipid metabolism is associated with activation of PPAR-α. In the skin, PPAR-α regulates sebum production, keratinocyte proliferation and differentiation, and barrier formation. In these studies we analyzed PPAR-α in HN2 injury and repair in mouse skin. The shaved dorsal skin of female CD-1 mice (8-12 weeks of age) was treated with HN2 (77 ng) and examined for expression of PPAR-α and markers of sebocyte and keratinocyte proliferation and maturation 1-5 days later. In untreated control skin only low constitutive levels of PPAR-α were evident in the epidermis and dermal appendages. One to two days post exposure to HN2, tissue injury was associated with a 10-fold upregulation of PPAR-α in the remnant sebaceous glands, hair outer root sheath (ORS) cells and basal epidermal cells. This effect was associated with a 5-fold decrease in expression of fatty acid synthase (FAS), a marker of sebocyte maturation, keratin-10, a marker of keratinocyte differentiation and proliferating cell nuclear antigen (PCNA) in sebocytes and basal cells. Three to five days post-HN2, a further increase in PPAR-α was noted at the wound edge, the neoepidermis, the interfollicular epidermis and the stratum corneum. This effect was associated with an increase in expression of the epidermal differentiation marker keratin-10 in the suprabasal epidermis and an increase in the numbers of basal cells expressing PCNA in the neoepidermis. High levels of PPAR-α mRNA and protein expression were observed. Taken together these data indicate that PPAR-α is altered following injury and during repair following exposure to HN2. We speculate that PPAR-α-targeted therapy may be beneficial in mitigating HN2-induced skin injury. Supported by NIH AR055073.

2095 Tertiary and Quaternary Oxime Reactivation of Sarin-, Soman-, and Paraoxon-Inhibited Acetylcholinesterase in Zebrafish Larvae

J. A. Koenig, T. L. Dao, R. K. Kan and T.-M. Shih. *US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.* Sponsor: J. Dillman.

When searching for effective organophosphorus (OP) nerve agent medical countermeasures, *in vitro* assays are used as the preliminary screen before moving into the *in vivo* rodent model. While the rodent model is an effective tool, it is laborious, expensive, and lacks high-throughput capabilities. The present study aimed to establish zebrafish larvae as a novel *in vivo* model for nerve agent exposure and treatment, specifically in screening for oxime reactivators, the class of compounds capable of actively rescuing nerve agent-inhibited acetylcholinesterase (AChE). Zebrafish possess high fecundity, are relatively inexpensive, develop quickly, and are an already well-established model in biomedical research. The LC50 values were determined for the nerve agents sarin (GB) and soman (GD), and the OP pesticide paraoxon at 30, 60, 90, and 120 min exposure times in six days post-fertilization (6 dpf) larval zebrafish. A time- and concentration-dependent mortality was observed, with GB and GD demonstrating greater toxicity than paraoxon. Zebrafish larvae were then exposed to the 60 min LC50 concentration for a time sufficient to produce maximum AChE inhibition (>98%) before oxime administration. The oxime compounds evaluated were the quaternary oximes 2-PAM and MMB-4, and the tertiary oxime MINA, with concentrations ranging from 25 µM to 400 µM. For GB, all three oximes demonstrated significant reactivation at the highest concentration, with 2-PAM, MMB-4, and MINA returning AChE activity to 23%, 22%, and 17% of control, respectively. For paraoxon, only 2-PAM and MMB-4 were significantly effective at the highest concentration, returning AChE activity to 55% and 34% of control, respectively. For GD exposure, all three oximes proved ineffective. These results correlate with previous rodent reactivation data and lend support to the use of zebrafish larvae as a high-throughput screen in the search for more efficacious oxime reactivators following nerve agent exposure.

2096 Absorption Kinetics and Blood Brain Barrier (BBB) Penetration by a Cyanide Antidote (CAX1) on a Mice Model

B. A. Mendenhall, L. Kiss, S. Crews, J. Lowry, V. Coronado, J. Ross, D. Brown and I. Petrikovics. *Chemistry, Sam Houston State University, Huntsville, TX.* Sponsor: I. Petrikovics.

Background: Cyanide is a rapidly acting toxic substance with primary target organs of brain and heart. It is a strong requirement from a cyanide antidote to act rapidly and efficiently. Methods: This study focuses on 1) the determination of the rate of absorption to the circulation 2) distribution to the brain after crossing the BBB when our antidote test molecule (CAX1) was administered intramuscularly to mice. To measure the test molecule concentration in blood and brain, analytical methods were developed using HPLC-UV and GC-MS-SPME respectably. Results: The absorption kinetics study indicated that the test molecule (CAX1) appeared in blood right after the intramuscular injection with the Tmax=5-15 mins and it was eliminating within two hours at the dose of 200 mg/kg in mice. It also appeared in brain rapidly, indicating a good BBB penetration. The Tmax in brain was 15 mins, and the rate of elimination from brain was significantly faster than from blood. Conclusions: These analytical methods proved to be usable for pharmacokinetics and organ distribution studies. The rapidly acting cyanide antidote CAX1 with its antidotal efficacy (reported later) shows a potential as a cyanide antidote. The study was funded by the Robert A. Welch Foundation at Sam Houston State University (x-0011) and the USAMRICD (Contract No.W911NF-11-D-0001).

2097 Peroxiredoxins are Molecular Targets for the Sulfur Mustard Analog Mechlorethamine in Human Keratinocytes

Y.-H. Jan[2], D. E. Heck[3], V. Mishin[4], R. P. Casillas[1], D. L. Laskin[4] and J. D. Laskin[2]. *[1]MRIGlobal, Kansas City, MO; [2]Environmental & Occupational Health, Rutgers School of Public Health, Piscataway, NJ; [3]Environmental Health Science, New York Medical College, Valhalla, NY and [4]Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Mechlorethamine (Bis(2-chloroethyl)methylamine, HN2), a bifunctional alkylating agent commonly used as a model compound to study sulfur mustard-induced injury, is known to cause oxidative skin injury. Peroxiredoxins (Prxs), important in antioxidant defense, cellular signaling, and tissue repair, are a family of peroxidases with a conserved cysteine (Cys) residue essential for reducing peroxides. Previously, we reported that HN2 predominantly targeted cysteine residues on the thioredoxin system resulting in enzyme inactivation, a process contributing to toxicity. In this study, we examined the effects of HN2 on Prxs. HN2 treatment was found to cause a concentration- and time-dependent inhibition of Prxs in human HaCaT keratinocytes. Immunoblot analysis of HN2-treated cells revealed selective cross-linking of Prx1, Prx3, and Prx6 forming higher molecular weight bands corresponding to dimers of Prx, which are not detected in untreated cells. Using recombinant human Prx1, the formation of protein cross-links and enzyme inhibition were found to be redox status-dependent; reduced enzyme was more sensitive to HN2 than oxidized enzyme. These data suggest that HN2 directly targets catalytic cysteine residue in Prx. LC-MS/MS analysis demonstrated that HN2 selectively alkylated cysteine and lysine residues on the enzyme, including peroxidatic Cys-52 and resolving Cys-173 in human Prx1, providing a mechanism for enzyme dimerization and inactivation in keratinocytes. These data demonstrate that peroxiredoxins are molecular targets for sulfur mustard vesicants. Inhibition of Prxs may be an important mechanism by which vesicants induce oxidative stress and tissue injury. Support: NIH grants AR055073, NS079249, ES004738, CA132624 and ES05022.

2098 A Novel Peptide Protects Against Nerve Agent Poisoning

U. Wormser[2], B. Brodsky[2], Y. Finkelstein[1] and E. Proscura[2]. *[1]Shaare Zedek Medical Center, Jerusalem, Israel and [2]The Hebrew University, Jerusalem, Israel.*

The currently available antidotes for treatment of organophosphate poisoning are atropine, oximes and anticonvulsants while pyridostigmine is a prophylactic measure. The present study demonstrates a marked antidotal activity of the novel peptide QVP against toxicity of nerve agents. Paraoxon administration (female ICR mice, 1.8mg/kg, sc) caused death within 20 minutes of exposure whereas no mortality was observed in animals received a single injection of the peptide (1mg/kg, iv) 8 days prior to the nerve agent intoxication. This effect was well correlated with the pronounced peptide-induced reduction of the inhibitory effect of paraoxon on brain acetylcholinesterase activity which was 4 fold higher in the QVP-treated mice than the control group. No differences between peptide-treated and control groups were detected in serum paraoxonase and butyrylcholinesterase activities. A remarkable protection was also observed at an interval of 9 days between QVP treatment and paraoxon exposure while shorter interval of 7 days caused a weak protective effect. It should be noted that QVP was the only countermeasure used along these experiments, namely, neither atropine nor oximes were employed. The long interval between QVP treatment and paraoxon exposure indicates for a potential peptide-induced brain factor that modulates acetylcholinesterase activity and confers protection against nerve agent toxicity. (This work was supported by HDTRA contract number 1-13-1-0041).

2099 Activation of Thioredoxin-Regulated Signaling in Human Lung Epithelial Cells by Nitrogen Mustard Vesicants

D. E. Heck[3], Y.-H. Jan[2], R. P. Casillas[1], D. L. Laskin[4] and J. D. Laskin[2]. *[1]MRIGlobal, Kansas City, MO; [2]Environmental & Occupational Health, Rutgers School of Public Health, Piscataway, NJ; [3]Environmental Health Science, New York Medical College, Valhalla, NY and [4]Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Nitrogen mustards (NM) are bifunctional alkylating agents commonly used in cancer chemotherapy and in models to study sulfur mustard-induced tissue injury. Exposure to these vesicants is known to cause oxidative tissue injury as a consequence of their ability to alkylate cellular macromolecules. The thioredoxin (Trx) system, which includes thioredoxin reductase (TrxR), Trx and NADPH, is a cellular disulfide reduction system important in antioxidant defense and cell growth control. Previously, we reported that mechlorethamine (HN2) selectively targeted the Trx system in lung A549 epithelial cells by cross-linking both Trx and TrxR forming intra-, inter-molecular, as well as Trx/TrxR complexed cross links. In these studies, we compared the actions of HN2 with chlorambucil (CHL) and melphalan (MEL) on the Trx system and Trx-regulated signaling pathways. Each NM caused a concentration- and time-dependent inhibition of the Trx system in A549 cells. HN2 was the most active followed by CHL and MEL. Inactivation of the Trx system with NM was correlated with the formation of high molecular weight Trx-TrxR cross-links and activation of Trx-regulated signaling pathways including p53 and p44/42. Using purified Trx and TrxR, protein cross-linking and enzyme inhibition by NM were found to be redox status-dependent. Reduced proteins were more sensitive to vesicants than oxidized proteins suggesting that these vesicants directly target catalytic residues in Trx and TrxR leading to enzyme inactivation. All three vesicants are structurally similar with reactive chloroethyl groups. However, they vary with respect to substitutions on aziridine; HN2 contains a methyl group while CHL and MEL contain phenylbutanic acid and phenylalanine, respectively. Modeling studies demonstrate that bulky phenyl groups on CHL and MEL decrease accessibility to alkylation sites on Trx and/or TrxR, resulting in decreases in protein cross-linking and enzyme inhibition. Taken together, our data demonstrate that the Trx system is a molecular target for NM vesicants. Accessibility of nitrogen mustards to alkylation sites on Trx and TrxR limits their ability to inhibit the Trx system. Support: NIH grants AR055073, NS079249, ES004738, and ES005022.

2100 Optimization of a Neurotoxicology Animal Model for Therapeutic Intervention Studies

W. M. Weber[2], J. D. McDonald[2], J. Lewine[3], M. Doyle-Eisele[2], R. Raulli[1], J. Laney[1] and E. Garcia[1]. *[1]Biomedical Advanced Research and Development Authority, Washington, DC; [2]Lovelace Respiratory Research Institute, Albuquerque, NM and [3]Mind Research Network, Albuquerque, NM.*

Sarin (GB) is a highly toxic nerve agent that functions by inhibiting acetylcholine esterase. Low doses of GB result in seizures, muscle fasciculation, pupil constriction, and weakness while high doses result in status epilepticus (status), full body convulsions and tremors, respiratory distress, loss of consciousness, and death. Treatments such as atropine and 2-PAM, both part of the DuoDote® Auto-Injector, can assist in overcoming the acute peripheral toxicity of sarin exposures. These kits do not prevent long term cognitive dysfunction, behavioral changes, or memory loss, which have been reported in patients surviving an acute nerve agent exposure. The goal of this program was to develop a model in F344 rats that can be used to evaluate therapeutic intervention strategies to reduce seizure and prevent long term neurological disorders. Animals were initially treated with 100 µg/kg of GB, the literature reported LD50 value, however, the supportive care regimen of atropine (3

mg/kg) and 2-PAM (25 mg/kg) at 1 minute post GB exposure afforded almost full protection of the animals; minimal seizure activity was noted. There were only sporadic findings of status. When the dose of GB was increased to 1.2x LD50 (120 µg/kg) an increase in seizure activity was noted but no status. To refine the model, a lower dose of atropine (2 mg/kg) was administered as well as evaluation of three forms of atropine (atropine, atropine sulfate, atropine methyl nitrate). Atropine methyl nitrate does not cross the blood brain barrier and therefore, as anticipated, increased seizure activity but still afforded the animals protection from the acute toxicity of GB. The number of animals entering status was highest when the dose of GB was increased to 1.4x LD50 with 66% of animals developing status and 83% of animals developing seizures. When midazolam (0.66 mg/kg) was administered at 40 minutes post sarin exposure, seizure activity was stopped in all animals; however, seizure activity returned approximately 4 hours later in those animals that initially reached status. This model will be utilized in future studies to evaluate anti-seizure agents at preventing long-term disorders. These studies were funded and approved by BARDA under contract number HHSO100201100004I/HHSO10033007T.

2101 14C VX Skin Penetration from Water *In Vitro*

C. H. Dalton, S. J. Graham and J. Jenner. *CBR Division, Dstl, Salisbury, United Kingdom.*

VX is a chemical warfare agent categorised as a nerve agent. The main route of absorption of liquid VX into the body is percutaneous penetration. Should a person become contaminated with VX, removal of VX from the skin surface would be attempted by either dry or wet decontamination. Wet decontamination involves the application of water (either with or without detergent) to wash away the agent. The wash-in effect sees an increase in percutaneous absorption after washing. Likely mechanisms to describe the effect have been ascribed to surfactant, hydration, acid/base, friction and artefact effects [1]. The current study considered whether the presence of water altered the percutaneous penetration of VX *in vitro*. Franz type static diffusion cells (diffusional surface area 2.54cm2) were used. Pig abdominal flank skin was used as a surrogate for human skin. The receptor fluid was 50% aqueous ethanol. 14C-VX and water were mixed immediately prior to skin application. The mixture was left in contact with the skin for the 24h study duration under occluded conditions. Receptor fluid samples were taken at regular intervals and 14C was measured by scintillation counting. Measured steady state penetration rates (Jss, µg cm-2 h-1) were 167 ± 88 (100% VX control), 185 ± 103 (90% VX 10% Water), 245 ± 175 (80% VX 20% Water), 341 ± 150 (50% VX 50% Water), 258 ± 93 (20% VX 80% Water) and 189 ± 71 (10% VX 90% Water). All values are mean ± standard deviation of n=8 individual animal replicates. Statistical significance (P <0.05, one way ANOVA with Dunnett's multiple comparison test) was measured between the Jss values for the VX control and 50% VX 50% Water. This study may be viewed as a worst case scenario study with both VX and water in contact with the skin for substantially longer than would be the case in reality. [1] Moody R P and Maibach H I. Skin decontamination: Importance of the wash-in effect. Food and Chemical Toxicology 44: 1783-1788 (2006). © Crown copyright 2015. Published with the permission of the Defence Science and Technology Laboratory on behalf of the Controller of HMSO.

2102 Acute Toxicity of Phorate Oxon in the Sprague-Dawley Rat by Oral Gavage

M. K. Brittain[1], M. C. Babin[1], T. H. Snider[1], D. A. Jett[3], G. E. Platoff[3] and D. T. Yeung[3]. [1]*BioMedical Research Center, Battelle, Columbus, OH;* [2]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD and* [3]*National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD.*

Phorate is an organophosphate (OP) systemic pesticide and aracicide used at 20% v/w strength in slow-release granules. Like other OPs, phorate is bioactivated to the more toxic form, in this case phorate oxon (PHO; O,O-diethyl S-[(ethylsulfanyl)methyl] phosphorothioate). Toxicity information is generally not available on PHO, and the objective of this study was to help fill a data gap on its acute oral toxicity in both sexes of the Sprague-Dawley rat. PHO was administered by one-time gavage as a 0.125 mg/mL solution in corn oil. In the first of two studies, the median lethal dose (LD50) was determined in each sex. Lethality was assessed at 24 hr after challenge. In the second study, sex and age effects on the toxicity of PHO at non-lethal levels were assessed among 10- and 16-week old subgroups. Pre-challenge and 24hr blood samples were collected via jugular venipuncture in sedated rats, and survivors' brain tissue was collected and cryopreserved. Blood and brain samples were processed and assayed for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities. The oral LD50 (95% confidence limit) was 0.88 (0.79, 1.04) mg/kg in males and 0.55 (0.46, 0.63) mg/kg in females with a common probit slope of 15. The males were significantly ($p < 0.0001$) more tolerant of oral PHO. From the second study, higher titers of blood AChE and BChE were present in female rats, and 10-week rats generally had lower levels. However, the rate of decreasing baseline-normalized activity levels in response to increasing PHO challenges was similar among all groups. In brain tissue, the BChE and especially AChE inhibition response to increasing PHO challenges in 10-week females was more pronounced than that in either older females or 10- and 16-week old males. Enhanced tolerance in male rats may be due to hepatic carboxylesterase titers that increase with age.

2103 Oxime Therapy to Provide 50% Survival in the Hartley Guinea Pig Against Organophosphorus Challenges

C. Wilhelm[1], T. Snider[1], B. Reed[1], M. Babin[1], G. Platoff[2], D. Jett[2] and D. Yeung[2]. [1]*Biomedical Research Center, Battelle, Columbus, OH and* [2]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD;* [3]*National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD.*

Organophosphorus (OP) compounds, including pesticides and chemical warfare nerve agents, represent a threat to the general population as possible weapons of terrorism or from accidental releases during transportation or at storage facilities. A quick-acting therapeutic regimen that is efficacious over a broad spectrum of OPs would benefit the civilian population. This oxime program assessed eight oximes administered by intramuscular (IM) injection against both nerve agents and pesticides. Challenges at twice the median lethal dose (LD50) were administered either subcutaneously (SC) or percutaneously (PC) in evaluations of efficacy, protective ratios, and median effective dose (ED50). The ED50 results presented are the top performing oximes as therapeutics against OPs delivered both via the SC and PC routes. Pre-hospital atropinized (0.4 mg/kg) male Hartley guinea pigs were administered GB, phorate oxon or VX subcutaneously with therapy a minute later by 2-PAM Cl, MMB4 DMS or HLö7 DMS. The same paradigm was used for the percutaneous OP exposure of VX, VR or phorate oxon with therapy (2-PAM Cl, MMB4 DMS, HLö7 DMS or obidoxime Cl2) provided at the onset of signs and administered in triplicate. All oximes evaluated in the SC study produced useful ED50 values. However, since high levels of oxime therapy can enhance lethality from SC doses of OPs, it is important to ensure that therapies are efficacious within a wide window of safety in order to avoid such toxic synergies. Against PC OP challenges, none of the oximes were significantly beneficial in terms of 24-hr survival to the extent that probit analysis could resolve a dose/survival relationship. Due to slow release of OP from skin depot, dermal exposures will require sustained, although somewhat reduced, therapy administration over protracted periods for successful treatment.

2104 Efficacy of Pyridostigmine Bromide as a Pretreatment Against Nerve Agents in Guinea Pigs

J. Harvilchuck, J. Richey, N. Niemuth and J. Plahovinsak. *Battelle, Columbus, OH.*

Pyridostigmine bromide (PB) has been shown to be an effective pretreatment against soman (GD) toxicity when used in combination with rapid administration of atropine and pyridine-2-aldoxime methylchloride (2-PAM Cl) and is currently approved for use by the US military. This study was designed to evaluate the efficacy of PB as a pretreatment against tabun, sarin, cyclosarin, soman, VX and Russian VX when administered with standard nerve agent therapy (atropine, 2-PAM Cl, midazolam), in an unanesthetized male Hartley guinea pig model. Primary endpoints for this study were 8, 24, 48 and 72 hour survival and clinical observations to include Quality of Life (QOL). Guinea pigs were pre-treated with 0.026 mg/kg PB or saline approximately 30 minutes prior to challenge. Animals were then challenged with nerve agent [between 2x and 5x LD50 subcutaneous] one minute prior to IM administration of atropine (0.4 mg/kg), 2-PAM (25.7 mg/kg) and midazolam (0.66 mg/kg). Thirty minutes following challenge, surviving animals in designated groups were administered additional atropine (1.3 or 2.6 mg/kg; IM). QOL scores were calculated from observations taken at 10 time points including prior to PB administration through 72 hours post challenge. For animals challenged with nerve agent, the addition of PB pretreatment increased survival and lengthened the time to death when compared to animals that did not receive PB (with or without additional atropine). The lowest QOL scores (closest to normal) at 72 hours post-challenge were observed in animals that received PB, both with and without the additional atropine. PB was most effective when used in conjunction with a high dose of additional atropine. The addition of PB did delay

the time to death of the animals, although not significantly for most groups, possibly allowing time for further treatment interventions to improve survival. This work was supported by funding from Contract No. SP0700-00-D-3180 and developed in collaboration with Medical Countermeasures Systems, US Army Medical Research Institute for Chemical Defense and the Defense Threat Reduction Agency. The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the US Government. Distribution Statement A: Approved for public release; distribution is unlimited.

2105 Larval Zebrafish as a Model for Discovering Therapeutics for Chemical Threat Agent-Induced Seizures

S. Bandara[1], D. Feldman[2], G. Miller[1], C. Lossin[2] and P. Lein[1]. *[1]Molecular Biosciences, School of Veterinary Medicine, University of California-Davis, Davis, CA and [2]Neurology, School of Medicine, University of California-Davis, Sacramento, CA.*

Zebrafish (*Danio rerio*) have recently emerged as models of epilepsy, as demonstrated by the ability of pentylenetetrazole (PTZ) to trigger seizure-like behavior in these animals. Here we show that zebrafish larvae also exhibit seizures following acute intoxication with diisopropylfluorophosphate (DFP) or tetramethylenedisulfotetramine (TETS), both highly toxic chemicals that cause seizures via diverse mechanisms. At 5 days post fertilization (dpf), tropical 5D wildtype zebrafish were placed in 96-well plates (1 fish per well) and exposed to DFP, TETS or the positive control PTZ added to fish water. The resulting behavior/movement was recorded for 20 min post-exposure using the Noldus automated tracking system. Exposure to DFP or TETS resulted in a concentration-dependent stereotypical sequence of behavioral changes progressing from hyperactivity (Stage 1) to whirlpool-like increased swimming (Stage 2) culminating in clonic-like convulsions/immobility (Stage 3). Extracellular field potential recordings obtained using a 1-micron glass electrode filled with 2M NaCl placed in the optic tectum of 7 dpf zebrafish embedded in agarose revealed unique extracellular spiking patterns consistent with seizure activity following acute exposures to DFP or TETS. Post-exposure treatment with the anticonvulsants diazepam or midazolam attenuated seizure behavior and diminished the extracellular spike activity in these animals. These results demonstrate that DFP and TETS elicit behavioral and electrophysiological changes in larval zebrafish similar to those reported in rodent models of acute DFP or TETS intoxication. Thus, zebrafish present an exciting new opportunity for streamlining the process for discovering novel medical countermeasures for these chemical threat agents. Supported by NIH CounterACT grant # NS079202.

2106 Substrate and Inhibitor Kinetics of Free and Copolymer-Complexed Recombinant Human Butyrylcholinesterase

K. Poindexter[4], J. Liu[4], L. G. Sultatos[3], N. Flynn[4], J. D. Ramsey[4], S. Hartson[4], A. Ranjan[4] and C. Pope[4]. *[1]Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK; [2]Chemical Engineering, Oklahoma State University, Stillwater, OK; [3]Department of Pharmacology, Physiology and Neuroscience, New Jersey Medical School, Rutgers University, Newark, NJ and [4]Physiological Sciences, Oklahoma State University, Stillwater, OK.*

Use of human butyrylcholinesterase as a bioscavenger for organophosphorus (OP) toxicants is hindered by its limited availability and rapid clearance. Glutaraldehyde crosslinked, electrostatic complexes made from recombinant human BChE (rhBChE) and copolymers of poly-L-lysine (PLL) and poly(ethylene glycol) (PEG) may have benefits over the use of the free enzyme as bioscavengers. We previously reported that complex formation led to variable loss in enzyme activity (up to about 50%), depending on the size and relative concentration of the copolymer. Surprisingly, free enzyme and the copolymer-BChE complexes showed similar concentration-dependent inactivation of paraoxon *in vitro*. Moreover, paraoxon had similar potency *in vitro* (IC50 4-10 nM, 37^{0}C, 20 min) against the free and copolymer-complexed enzyme. We conducted kinetic analyses to determine if copolymer-BChE complex formation altered the interaction between BChE and either substrate or inhibitor. Free enzyme or a copolymer-BChE complex (using high [30-70 kDa], medium [15-30 kDa], or low [4-15 kDa] molecular weight PLL; all with 10:1 PEG:PLL) was incubated with a range of concentrations of butyrylthiocholine (10-1,000 μM) to determine Km and Vmax using non-linear regression. We then evaluated time-dependent inhibition of BChE activity using a range of paraoxon concentrations (50-500 nM, 37C^0). Primary kinetic plots of residual activity vs time and slopes of primary plots vs inhibitor concentration were used to estimate Ki. As expected, there was a significant reduction in Vmax (30-45%) with the copolymer-BChE complexes compared to free enzyme. In contrast, no significant changes in Km were noted (mean values, 119-194 μM). Ki values for paraoxon with the free and copolymer-complexed BChE were also similar (2.5-2.7 x 106 M-1 min-1). Together, our data suggest that complex formation between the copolymer and rhBChE may lead to altered catalytic hydrolysis of butyrylthiocholine but little effect on the interaction between the enzyme and an OP inhibitor. (Supported by DTRA grant HDTRA1-13-1-0021).

2107 Blood-based miRNA Biomarkers of VX Exposure

J. A. Gibbons[1,2], R. Balog[3,4], C. Rothwell[3], A. Melber[3], B. Benton[2], H. Hoard-Fruchey[3] and J. W. Sekowski[2]. *[1]Excet, Inc., Springfield, VA; [2]US Army Edgewood Chemical Biological Center, APG-EA, MD; [3]US Army Medical Research Institute for Chemical Defense, APG-EA, MD and [4]US Army Reserve, Apg-ea, MD.* Sponsor: S. Hulet.

Chemical warfare agents (CWAs) continue to be a threat to military and civilian populations at home and abroad during the war against terrorism. Exposure to chemical nerve agents such as VX is known to disrupt cholinergic pathways, resulting in a cascade of toxic effects relative to the exposure level, including miosis, excessive secretions, convulsions, seizures, and death. While much is known about the main effects of nerve agents, there remains a paucity of information about the molecular effects. MicroRNAs are a relatively novel epigenetic regulator of gene expression. Each mature miRNA can potentially regulate hundreds of mRNA targets, and therefore affect multiple disparate biological functions. This investigation is aimed at discovery and validation of novel biomarkers of VX exposure and effect. Whole blood from adult male Sprague-Dawley rats was collected at 1, 2, 4, 8, and 24 hours after exposure to 0.4, 0.7, or 1.0 x LD50 VX (i.v.). Mature miRNA expression for each sample was determined via microarray, and for pooled samples via quantitative PCR (qPCR). Microarray results identified 54 background-corrected miRNAs up- or down-regulated (≥ 1.2 or ≤ 0.8) at any time point and VX dose ($p < 0.05$, Student's t-test). For all VX doses, pro-apoptotic miRNAs were upregulated at 1 hour and 2 hour time points, but down-regulated at all later time points. At all time points, miRNAs involved in arrhythmias showed up-regulation at 0.4 LD50, but down-regulation at 0.7 and 1.0 LD50 doses. Biomarker panels of 4 or 5 miRNAs were determined for each time point from microarray results using a Support Vector Machine algorithm with a radial basis function kernel for exposed versus unexposed rats. Panel accuracy was 85% or greater at each time point, and showed no false positives at the 1 hour and 24 hour time points. Biomarkers for early time points are key for detect-to-treat applications, whereas later time points are useful in forensics. Finally, in anticipation of a potential diagnostic transition, microarray-derived biomarker panels were compared with qPCR results. Panel miRNAs correlated microarray and qPCR fold-change trends at early time points. The results of this study can be used to develop diagnostics for CWA exposure, as well as provide new insights into underlying biological function of these exposures.

2108 Impact of Rabbitpox Virus Infection on Oral Pharmacokinetics of Tecovirimat in New Zealand White Rabbits

M. Borges[1], M. Trinh[1], C. Lovejoy[2], M. Biswajit[2], A. Berhanu[2], D. Grosenbach[2], F. Koide[3] and M. Beliveau[1]. *[1]Certara Strategic Consulting, Montreal, QC, Canada; [2]Siga Technologies, Inc., New York City, NY and [3]Southern Research, Frederick, MD.*

Smallpox is still considered a serious bioterror threat even though naturally occurring smallpox has been eradicated worldwide. New Zealand White (NZW) rabbit infection with Rabbitpox virus (RPXV) has been proposed as a surrogate for testing antiviral drug efficacy against variola virus, the causative agent of human smallpox. The present study evaluated the pharmacokinetics (PK) of oral tecovirimat, an antiviral drug developed for the treatment of pathogenic orthopoxvirus infections, in uninfected NZW rabbits and the impact of a lethal RPXV infection on its PK. Three single doses of tecovirimat (40, 80 and 120 mg/kg), all of which provide significant protection from mortality, given to RPXV infected NZW rabbits at 4 days post-infection yielded similar PK as uninfected NZW rabbits. Mean tecovirimat AUC0-t, Cmax and Cmin ranged from 8547 to 16951 ng•h/mL, 699 to 1214 ng/mL and 151 to 456 ng/mL, respectively, prior to RPXV infection; and from 7905 to 18225 ng•h/mL, 603 to 1199 ng/mL, and 178 to 578 ng/mL, respectively, post-infection. Repeated doses of tecovirimat 40, 80 and 120 mg/kg QD for 7 days, yielded mean AUC0-t, Cmax and Cmin from 8974 to 24615 ng•h/mL, 653 to 1382 ng/mL, and 188 to 775 ng/mL, respectively. Following 14 once-daily doses post-infection at the same dose levels, mean AUC0-t, Cmax and Cmin values appeared to be slightly lower vs 7 once-daily doses. The accumulation ratio less than one, suggesting minimal accumulation after repeated dosing. Mean CL/F ranged from 36.8 to 51.3 L/h and mean

Vz/F ranged from 251 to 637 L following repeated dosing for 14 days. Mean tecovirimat AUC0-t, Cmax and Cmin following single and multiple doses of tecovirimat generally increased in a dose-proportional manner. No sex related trends were observed. Overall, the results of the study indicated a limited impact of RPXV infection on the pharmacokinetics of tecovirimat. This work was funded by Contract HHSO100201100001C from the Biomedical Advanced Research and Development Authority.

PS 2109 Effect of a Novel Brain-Penetrating Oxime Reactivator on Sarin Surrogate-Induced Changes in Inflammatory Factor Gene Expression in the Rat Piriform Cortex

M. B. Dail[2], E. C. Meek[2], C. A. Leach[2], H. W. Chambers[2] and J. E. Chambers[2]. [1]*Center for Environmental Health Sciences, Coll. Vet. Med., Mississippi State University, MS State, MS and* [2]*Dept. of Biochem., Mol. Biol., Entom., and Plant Path., Mississippi State University, MS State, MS.*

The nerve agent sarin stimulates the expression of inflammatory response genes in the rodent brain. Our lab has developed novel oxime acetylcholinesterase reactivators that penetrate the BBB in *in vivo* tests with nerve agent surrogates. A PCR array for rat inflammatory cytokines and receptors was used to examine changes in mRNA levels of 84 genes in the piriform cortex after exposure to a sarin surrogate, nitrophenyl isopropyl methylphosphonate (NIMP) and treatment with one of our substituted phenoxyalkyl pyridinium oximes. The hypothesis was that NIMP would increase mRNA transcription from inflammatory genes whereas treatment with the novel oxime would tend to counteract the effect of the surrogate and return levels closer to those seen in the vehicle control. Rats were administered either 0.325mg/kg NIMP or the vehicle (DMSO) sc. At 1 hr. post-NIMP exposure rats were administered 146mmol/kg oxime (human equivalent dosage) in Multisol im. Rats were sampled 2 hrs. later. Total RNA was extracted from the piriform cortex and cDNA was prepared then added to Qiagen's RT2 qPCR Mastermix for analysis using their PCR array for rat inflammatory cytokines and receptors. Data from vehicle, NIMP, and NIMP plus oxime were compared via Qiagen's GeneGlobe Data Analysis Center. A generalized repression of gene expression was observed following NIMP exposure. CT values less than 25 comprised 30% of the vehicle results but only 25% of NIMP's. Oxime treated had levels closer to those of vehicle with CT values below 25 comprising 27%. NIMP stimulated gene expression of Ccr3, Ccl3, Tnfsf11, Il6r and Cxcr1 but in the oxime treated tissue these genes showed repressed expression levels similar to those of the vehicle. The data suggest that the novel oxime crossed the BBB and altered gene expression in a potentially neuroprotective manner. (Partial support from NIH UOINS083430).

PS 2110 A Hairless Guinea Pig Model of Sulfur Mustard Vapor-Induced Dermal Partial Thickness Injury

F. Reid[2], D. Barillo[1], C. R. Croutch[2], T. Culley[2], W. Sosna[2] and J. Roseman[2]. [1]*Disaster Response/Critical Care Consultants LLC, Mt Pleasant, SC and* [2]*Medical Countermeasures, MRIGlobal, Kansas City, MO.*

Rationale: Sulfur mustard (SM) penetrates the skin quickly producing a delayed injury ranging from erythema to third degree or deeper injuries with bullous blisters. The current treatment for SM exposure is supportive care. Testing of therapeutics and medical devices designed to improve the healing time, protect the lesion, and reduce or eliminate the chronic injury seen after a SM vapor exposure require a reliable standard small-animal model. Methods: 16 Hartley hairless guinea pigs (HGP) weighing ~425 gm and ~7 weeks of age were used to create two depths of dermal injury, superficial dermal (SD) and moderate dermal depth (DD) on the dorsum of HGP. Each HGP had four dorsal sites (2 sites per side of the dorsal midline) exposed to neat sulfur mustard vapor at various exposure times (5-60 min). A control and each wound site were evaluated (Pre-injury Study Day 0, post-injury Study Day 1 and 2) for modified Draize score, transepidermal water loss (TEWL), ultrasound, digital images, ballistometry, and colorimetry. At 48 h post-exposure, HGPs were euthanized to collect skin punch biopsies for histopathology. Results: A time dependent dose-response of increasing depth of injury and wound severity by 48 h post-SM exposure was demonstrated. Dermal exposure of sulfur mustard for 5-60 minutes resulted in microscopic changes consistent with human skin exposure (microvesicles, ulceration, and degeneration/necrosis). Erythema and necrosis scores, biomechanical measurements, and histopathology reflected these results ($p \leq 0.05$) when comparing sham vs. increasing exposure time groups. The microvesicles, total degeneration/necrosis, and dermal necrosis assessments in the HGP were used to define a 10 minute (~ 150 µ depth) and a 20 minute (~420 µ depth) cap time as optimal for production of superficial and deep dermal wounds. Conclusions: A reproducible small-animal model of superficial and deep dermal sulfur mustard vapor burns was developed for use in further military and civilian research.

PS 2111 Blood and Tissue Silver Levels Following Application of Silver-Based Dressing to a Chemical Burn Wound

D. Barillo[1], C. R. Croutch[2], F. Reid[2], T. Culley[2], W. Sosna[2] and J. Roseman[2]. [1]*Disaster Response/Critical Care Consultants LLC, Mt Pleasant, SC and* [2]*Medical Countermeasures, MRIGlobal, Kansas City, MO.*

Rationale: Silver ion in antimicrobial and silver-based dressings have been used in burn care for over 60 years. Recently developed silver dressings are designed to be left in place for 7 days. We examined wound, blood and tissue levels of silver ion following long term dressing placement over a dermal chemical burn injury. Methods: Superficial and moderate depth partial-thickness chemical burns were created in 16 hairless guinea pigs (4 sites/animal) and 16 Gottingen minipigs (8 sites/animal). These were debrided by laser treatment or mafenide acetate wet dressings and then covered with commercially available silver-based burn wound or calcium alginate dressings. Dressings were changed weekly until wound healing to maximum of 60 days. Wound, blood (pre- and post-wound induction), liver, kidney and spleen were harvested and silver assay performed using ICP-MS with an LOD down to 0.02 ppb. Results: Blood silver levels were < 0.003 µg/g and kidney levels were < 0.03 µg/g in all 32 animals tested. Liver levels were <0.05 µg/g in all animals and < 0.02 µg/g in 24 of 32 samples. Spleen levels were <0.05 µg/g in all animals and < 0.02 µg/g in 24/32 samples. For blood, liver, spleen and kidney, there were no species or treatment differences. Dermal punch biopsies from the wound site resulted in silver levels that ranged from < 0.07 µg/g to 19.5 µg/g and overall were higher in pigs than in hairless guinea pigs. Highest levels were seen in laser-debrided deep dermal porcine wounds (mean = 9.7 µg/g). Burn dressings produced higher wound silver levels than calcium alginate dressings. It is significant to note that while burn wound dressings were removed in some models/cohorts as early as Study Day 23, skin punch biopsies were not collected until at least Study Day 63 and silver was still detected in these samples over 40 days later. Conclusion: Silver-based burn dressings demonstrate the ideal toxicological characteristics of elevated silver ion levels at the directed site of use, but with no systemic effects.

PS 2112 Modulation of Wound Repair by Matricellular Proteins in Mustard Exposed Skin

Y.-C. Chang, R. Hahn, H.-Y. Chang, P. Zhou, M. Gordon and D. Gerecke. *Pharmacoloty and Toxicology, Rutgers University, Piscataway, NJ.* Sponsor: D. Gerecke.

Sulfur mustard (SM) and its analog nitrogen mustard (NM) are blistering agents. The dermal toxicity of these includes separation of the epidermis and dermis, severe inflammation and delayed wound healing. SM crosslinks many proteins and other skin molecules and leads to cell adhesion alteration which may contribute to prolonged wound healing. In normal adult skin, expression of the matricellular proteins (MPs) is limited. After skin injury, upregulation of MPs helps modulate cell-matrix interaction that leads to proper wound repair. Sequential changes of MPs such as osteonectin (SPARC), SPARC-like 1 (Hevin), and tenascin-C (TNC) and laminin 332 (LM332 or LM5), a basement membrane (BM) marker, were examined in NM exposed skin of hairless (SKH1) mice. Biopsies were taken at 1, 3, 7 and 10 days post exposure. H & E histology showed epidermis-dermis junction separation at 1 day. Epidermal hyperplasia and infiltration of inflammatory cells in the dermis occurred at 3 days, with a severe inflammatory response at 7 days, and persistent skin wounds by 10 days. Immunofluorescence (IF) studies of untreated skin showed a continuous staining pattern for LM5 in the BM zone with minimal staining of SPARC, Hevin, and TNC. At day 1, LM5 expressed discontinuously with strong expression of SPARC and basal expression of Hevin in the epidermis. TNC was primarily in the dermis near the BMZ. By day 3, staining of LM5 appeared multi-layered in the BMZ, Hevin localized to the hyperplastic epidermis while TNC was near BMZ and extended to deep in the dermis. Co-expression of LM5/Hevin, and LM5/TNC were visible in some basal epidermis cells and BMZ, respectively. Hevin and TNC continued to express strongly by day 7. By day 10, Hevin staining was persistent throughout the hyperplastic epidermis. LM5 and TNC expression was strongest in the BMZ although the TNC expression was behind the leading edge. The TNC expression was verified by QPCR and WB data. Overall, MPs persistently accumulated in NM injured skin and may play a role in delayed wound repair. Supported by ES005022, EY09056, and NIAMS U54AR055073

2113 Attenuation of Nitrogen Mustard (NM) Induced Pulmonary Injury and Fibrosis by Inhibitors of Tumor Necrosis Factor (TNF)α and Reactive Oxygen and Nitrogen Intermediates

R. Malaviya[3], V. Sunil[3], A. Venosa[3], K. Vayas[3], L. Hall[1], N. D. Heindel[2], C. J. Lacey[2], J. D. Laskin[3] and D. L. Laskin[3]. *[1]Janssen Research and Development, Raritan, NJ; [2]Lehigh University, Bethlehem, PA and [3]Rutgers University, Piscataway, NJ.*

NM is a vesicant which causes severe lung damage leading to fibrosis; this is associated with oxidative and nitrosative stress and inflammation. We previously demonstrated that the proinflammatory cytokine, TNFα and the nitric oxide generating enzyme, inducible nitric oxide synthase (iNOS), play key roles in the pathogenic response to NM. Herein we analyzed the effects of blocking TNFα in combination with reactive oxygen and nitrogen species on NM-induced lung injury. Treatment of rats with anti-TNFα antibody (15 mg/kg, i.v., 1x), the iNOS inhibitor, 1400W (50 mg/kg, i.p., 1x/d), or the antioxidant, N-acetylcysteine (NAC, 150 mg/kg, i.p., 1x/d) 30 min after NM (0.125 mg/kg, i.t.) reduced lung injury and inflammation, measured by bronchoalveolar lavage (BAL) cell and protein content and histology. NM-induced increases in expression of the oxidative stress marker, heme oxygenase (HO)-1 was also reduced, along with macrophage expression of cyclooxygenase (COX)-2, iNOS and TNFα, markers of a proinflammatory/cytotoxic M1phenotype; Ym-1 expression, a marker of profibrotic macrophages, was also reduced. Whereas the combination of anti-TNFα antibody and 1400W was more effective in reducing oxidative stress and suppressing proinflammatory and profibrotic macrophages than 1400W alone, the combination of anti-TNFα antibody and NAC was less active. Treatment of rats with NAC and 1400W or anti-TNFα antibody, 1400W and NAC down regulated proinflammatory and profibrotic macrophages; however, markers of lung injury were unchanged. These data demonstrate that reactive oxygen and nitrogen species and TNFα play roles in acute injury induced by NM, however, combined inhibition of these inflammatory mediators only marginally improves pathologic changes induced by this vesicant. Supported by NIH Grants HL096426, AR055073, ES004738 and ES005022.

2114 Assessing Novel Adenosine Treatment Strategies for Neuroprotection After Soman Exposure in a Rat Model

T. Thomas[1] and T.-M. Shih[2]. *[1]US Army Research Laboratory, Aberdeen Proving Ground, MD and [2]US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*
Sponsor: J. Dillman.

The brain-damaging effects of nerve agents are difficult to treat with current medical countermeasures. We recently reported that immediate central adenosine (ado) receptor (AR) stimulation with the ado A1 agonist (6)-cyclopentyladenosine (CPA) improved survivability and minimized neuropathology after soman intoxication. The goal for this study was to further explore ado's therapeutic applications and avoid peripheral side effects. We first investigated the neuroprotective efficacy of intracerebroventricularly (ICV) delivered CPA (700 µg) when given 20 min after the onset of soman-induced seizure. Delayed CPA treatment terminated seizure and protected against neuropathology with statistical significance, suggesting CPA has comprehensive protective capabilities. While ICV injection is a direct method to assess neuroprotection potential, it is not a clinically relevant route of administration. Toward developing a fieldable treatment strategy, the efficacy of systemic CPA injections was then tested. Results showed that systemic CPA co-administered with the peripherally acting AR antagonist 8-(p-sulfophenyl) theophylline (8-SPT) prevented soman-induced seizure while minimizing CPA's cardiovascular side-effects. Data suggest that sufficient quantities of CPA can reach the brain after a systemic injection and that its side-effects can be reduced. Therefore, ado-based treatments have the potential for clinical translation. One central side-effect of ado agonists is sedation. To overcome this, we investigated if the centrally acting AR antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) could reverse CPA-induced sedation. DPCPX (5 mg/kg, ip) given 3 hours after CPA (700 µg, ICV) reversed sedation and returned consciousness to the rats; however, the rise in brain activity was exacerbated, and seizure activity was induced. The level of excitotoxicity was significant as many animals developed neuropathology scores similar to those of animals exposed to soman. Future experiments will investigate if lower doses of DPCPX or alternative antagonists can safely reverse CPA-induced sedation. In conclusion, the results from this study suggested that both peripherally and centrally delivered ado agonists have significant therapeutic benefits for acute and delayed treatment of nerve agent poisoning, and it is possible to manage their side-effects.

2115 Efficacy of Dexamethasone, Doxycycline and Silibinin in Reversing Nitrogen Mustard-Induced Corneal Injury

D. G. Goswami[2], N. Tewari-Singh[2], R. Kant[2], C. Agarwal[2], D. Kumar[2], D. A. Ammar[2], J. M. Petrash[2] and R. Agarwal[2]. *[1]Department of Ophthalmology, University of Colorado Denver, Aurora, CO and [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Exposure to vesicating chemical warfare agent sulfur mustard (SM) causes severe injuries to the skin, eye and respiratory system. The eye tissue is particularly more sensitive to SM exposure causing an immediate incapacitation. The injury results in a brief asymptomatic phase followed by erythema and irritation, which could lead to temporary blindness. Despite the severity of the SM-induced ocular injuries, effective therapeutic interventions are not yet available. In our previous studies using nitrogen mustard (NM), an analog of SM, we have shown that NM induces potent corneal injury similar to SM involving DNA damage, inflammation, vesication and neovascularization in rabbit corneal culture and corneal epithelial cells. In the present study, we evaluated the therapeutic potential of an anti-inflammatory steroid dexamethasone (0.2%), an antibiotic and MMP inhibitor doxycycline (0.01%) and a non-toxic natural flavanone silibinin (0.02%) in reversing NM-induced corneal injury. The excised rabbit corneas were exposed to NM (100 nanomoles) for 2 h, washed with PBS and 10 µL of the test agents was added immediately (0 h) or 2 h after NM washout followed by a 24 h culture. Dexamethasone, doxycycline and silibinin treatment post NM-exposure led to a significant reversal (11% to complete) in NM-induced epithelial degradation, epithelial-stromal separation and apoptotic cell death. Significant reduction (11–45%) in NM-induced COX-2, VEGF and MMP-9 expression was also observed upon treatment with these agents. The effect of the drugs was more pronounced when added immediately after the NM washout compared to when added after 2 h. In immortalized human corneal epithelial cells, a decrease in cell death and a decrease in the expression of COX-2 and MMP-9 was observed upon treatment with 5 µM doxycycline, 10 µM dexamethasone or 20 µM silibinin, after 0 or 2 h post NM (5 µM) exposure. Results from this study show a beneficial effect of these drugs in reversing NM-induced corneal manifestations. This warrants further efficacy study of these agents in other vesicating agents, especially SM-induced corneal injury model towards the development of safe and effective therapeutics against vesicants-induced ocular injuries.

2116 Corneal Injury Following Ocular Exposure to Sulfur Mustard Vapor: Clinical, Histopathological and Molecular Endpoints

N. Tewari-Singh[3], D. G. Goswami[3], R. Kant[3], D. Kumar[3], D. A. Ammar[3], J. M. Petrash[3], R. W. Enzenauer[3], C. R. Croutch[1], R. P. Casillas[1] and R. Agarwal[3]. *[1]MRIGlobal, Kansas City, MO; [2]Department of Ophthalmology, University of Colorado Denver, Aurora, CO and [3]Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Sulfur mustard (2,2'-dichloroethyl sulfide; SM) is an alkylating chemical warfare agent, which causes debilitating injury to the ocular tissue, especially the corneal layer. Importantly, ocular tissue is almost ten times more sensitive to vesicant exposure than skin with devastating short- and long-term injuries including inflammation, corneal opacity, neovascularization (NV), ulceration and impaired vision. Since decontamination and available therapies only provide microenvironment conducive for healing of ocular tissue following SM exposure, there is lingering need for the development of effective targeted therapies. A number of studies have been conducted to study the pathogenesis of SM-induced ocular injury and test treatment modalities. However, mechanistic aspects of acute and chronic corneal complications remain to be delineated for the development of effective therapies. Hence, the present 28 day study was designed to develop a relevant New Zealand white rabbit ocular vapor SM exposure model and establish quantifiable clinical, histopathological and molecular endpoints of injury and healing. Using a more consistent and efficient exposure system, right eye of each anesthetized rabbit was exposed to 390-420 µg/L SM for 5 or 7 min and left eye was exposed to dilution air, which served as control. Clinical observations and scoring showed a SM dose-dependent increase in corneal opacity which was maximal at 3 days post-exposure. Corneal ulceration was maximal at 1 and 3 days post-SM exposure for 5 and 7 min, respectively. Corneal thickness, epithelial-stromal separation and epithelial degradation were maximal at 7 day post-SM exposure for 5 and 7 min. SM-induced corneal NV was observed at both the exposure times after 3 days post-exposure. Significant SM-induced increase in proteolytic and angiogenic mediators MMP-9 and VEGF was observed

within 1 day post-exposure, whereas inflammatory mediator COX-2 expression was significantly elevated starting at 3 days post-exposure. The ongoing studies in this model will help in further understanding the pathways involved in SM-induced corneal injury, and in identifying and testing effective treatment options.

2117 Activity-Based Protein Profiling Suggests Secondary Mechanisms of VX Intoxication

J. W. Sekowski[4], D. Carmany[1], A. Walz[4], F.-L. Hsu[4], F. Berg[4], D. Noort[2], R. Van Den Berg[2], J. A. Gibbons[1] and T. Glaros[4]. *[1]Excet, Inc., Springfield, VA; [2]The Netherlands Organization, Rijswijk, Netherlands; [3]BioSciences, US Army ECBC, APG-EA, MD and [4]Chemistry, US Army ECBC, APG-EA, MD.* Sponsor: S. Hulet.

Traditional chemical warfare agents continue to be a global threat. Exposure to organophosphate (OP) nerve agents, such as VX, is known to disrupt cholinergic pathways, resulting in increased acetylcholine (ACh) and a dose dependent cascade of toxic effects from miosis to death. While much is known about the main toxicity pathway, there remains a paucity of information regarding secondary mechanisms. The competitive activity-based protein profiling (ABPP) assay used in this work employed a biotin-labeled VX probe to enrich for VX binding proteins in cellular lysates from rat heart, liver, kidney, diaphragm and brain. Enriched peptides were eluted from protein-VX probe-streptavidin bead conjugates by trypsin digestion, and after TMT isotopic labeling were identified via quantitative mass spectrometric analysis. This assay specifically enriched a number of proteins, many previously reported to bind to other OP chemicals. A subset were not previously reported to interact with VX. Many of the proteins binding in four or five of the tissues tested were members of the metabolic serine hydrolase class, including energy metabolism (e.g. monoglyceride lipase), xenobiotic metabolism (e.g. carboxylesterases), and signaling molecule metabolism (e.g. platelet activating factor acetylhydrolase). Other classes included carrier, membrane, motor, and cytoskeletal proteins. VX inactivation of some of the identified proteins can lead to multiple effects, depending on tissue type. For example, VX binding of the L-tryptophan metabolizing enzyme, arylformamidase (AFMID), is predicted to lead to a reduction in product energy output (NAD(H) and NADP(H)) in all tissues; while the reduction in all its metabolites would reduce kynurenic acid (KYA), an endogenous inhibitor of glutamate NMDA receptors in the brain and nicotinic ACh receptors in muscle. Thus, a reversal of VX binding to AFMID and restoration of KYA could have both global and tissue specific toxicity dampening effects. Ongoing and future work stemming from this study includes: 1) validation of novel protein-agent adducts in blood useful for detect-to-treat or forensic detection of exposure, and 2) validation of the agent binding proteins common in multiple organs which may be potentially useful in the development of adjuvant therapeutic strategies.

2118 Protective Role of Surfactant Protein-D in Nitrogen Mustard Induced Lung Toxicity

V. Sunil, K. Vayas, J. Cervelli, M. Goedken, R. Malaviya, J. Laskin and D. Laskin. *Rutgers University, Piscataway, NJ.*

Nitrogen mustard (NM) and related alkylating agents are potent vesicants that target the lung, causing inflammation and fibrosis. Evidence suggests that alveolar macrophages contribute to the pathogenic response. Surfactant Protein (SP)-D is a pulmonary collectin known to suppress alveolar macrophage activity. In the present studies, we analyzed the effects of loss of SP-D on NM-induced lung toxicity. Wild type (WT) and SP-D-/- mice were treated with PBS or NM (0.08 mg/kg) intratracheally. Bronchoalveolar lavage (BAL) and lung tissue were collected 14 d later. Treatment of WT mice with NM resulted in perivascular inflammation and increases in BAL cell and protein content, indicating alveolar epithelial injury. Loss of SP-D resulted in a significant increase in the sensitivity of the mice to NM; thus, perivascular inflammation was more severe and there was evidence of foamy macrophages, perivascular edema, bronchiolar epithelial hypertrophy and hyperplasia, bronchiolar alveolar hyperplasia, bronchiectasis, fibrosis and emphysema. NM-induced oxidative stress, as assessed by hemeoxygenase-1 expression by alveolar macrophages, was also increased. Enlarged and highly vacuolated profibrotic Ym-1+ and mannose receptor-1+ alveolar macrophages were also present; in contrast, cyclooxygenase-2+ proinflammatory macrophages were decreased. These data indicate that SP-D plays an important role in protecting against NM-induced pulmonary toxicity. Elucidating mechanisms regulating macrophage activity following NM exposure may be important in developing therapeutics to treat vesicant-induced lung injury. Support: NIH AR055073 and ES005022.

2119 Identification of Differentially Expressed Proteins in Inferior Colliculus During Acute Hydrogen Sulfide Exposure

W. K. Rumbeiha[3], D.-S. Kim[3], P. Anantharam[3], A. Hoffmann[1], M. L. Meade[1], J. M. Gearhart[1], E. M. Whitley[2] and B. Mahama[3]. *[1]Henry M Jackson Foundation, Wright Patterson Air Force Base, Dayton, OH; [2]Pathogenesis, LLC, Gainesville, FL and [3]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA.*

Hydrogen sulfide (H2S) is a gaseous molecule produced endogenously and plays important roles in many physiological functions. Nonetheless, acute exposure to high concentration of H2S causes severe damage in brain and induces long-term neurological disorders. The cellular and molecular mechanisms underlying the H2S-induced dysfunction of central nervous system have not yet been clearly elucidated. To better understand the mechanisms of H2S-induced neurodegeneration, we used the broad-spectrum proteomic analysis to search for key molecular changes in H2S-induced neurotoxicity. C57 black mice were exposed by whole body inhalation to 310 ppm of H2S for 1, 2 or 4 days for 40 min in the first day and 15 min in subsequent days. The hydrogen sulfide-treated group showed behavioral motor deficits and developed lesions in inferior colliculus (IC) in the brainstem. The inferior colliculus was dissected at the terminal endpoints and used in proteomic analysis. Tandem mass tags (TMT) liquid chromatography mass spectrometry (LC-MS/MS) quantitative proteomics was applied for protein identity and quantitation. Quantitative real-time PCR was used for validation of resulting key proteins. We identified 3883, 3928, and 3843 differentially expressed proteins which were matched with HGNC identifier for 1, 2, and 4 days of H2S treated group, respectively. Database for Annotation, Visualization and Integrated Discovery was used to analyze functional gene ontology of statistically significant and distinctly differentially expressed proteins. Hydrogen sulfide treated group showed several dysregulated biological processes. Taken together, acute exposure to H2S-induced neurodegeneration in IC, and proteome in degenerated IC was analyzed using TMT LC-MS/MS. The obtained proteomic data may provide important clues to elucidate H2S-induced neurological disorders.

2120 Therapeutic Efficacy of Cobinamide for Treatment of Hydrogen Sulfide-Induced Neurotoxicity

P. Anantharam[4], E. M. Whitley[1], D.-S. Kim[4], D. Shao[4], M. Langley[4], B. Mahama[4], A. Kanthasamy[4], G. Boss[3] and W. K. Rumbeiha[4]. *[1]Pathogenesis LLC, Gainesville, FL; [2]Biomedical Sciences, Iowa State University, Ames, IA; [3]Department of Medicine, University of California, San Diego, San Diego, CA and [4]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA.*

Hydrogen sulfide (H2S) is a colorless and highly neurotoxic gas, and has been identified as an environmental pollutant with potential for misuse in chemical terrorism. Exposure to acute high dose of H2S can lead to acute death and neurological sequelae. Currently, there is no cure for H2S-induced neurotoxicity. The objective of this study was to evaluate the efficacy of cobinamide, a novel drug, as an antidote to H2S-induced neurotoxicity. C57 black mice were divided into 3 groups. Negative control group received normal breathing air. The other 2 groups were exposed to 310 ppm H2S by whole body inhalation for 40 minutes on day 1, followed by 15 min/day for 6 days and either given cobinamide (100 mg/kg bw) or 0.9% saline intramuscularly. A functional observation of battery was used to assess clinical signs during H2S exposure. Behavioral and histopathological changes were additional endpoints assessed. During exposure, cobinamide significantly reduced the number of mice with seizures and "knockdowns" compared to the H2S treated group. Cobinamide significantly reduced H2S-induced weight loss ($p < 0.05$). Cobinamide treated mice performed significantly better on the behavioral tests, significantly increased the time per trial on the Rotarod, and improved horizontal and vertical activity in the open field tests, compared to the H2S mice treated with saline ($p<0.05$). Histopathological changes were systematically assessed by a scoring system that accounted for loss of neurons, activated microglia, apoptotic bodies, and neuropil vacuoles. Using these histological endpoints, cobinamide significantly protected against H2S-induced neurodegeneration ($p<0.05$). Overall, these results suggest that cobinamide is neuroprotective, given before H2S exposure, and is a promising antidote. Ongoing work characterizes additional biomarker of H2S-induced neurotoxicity, and explores potential use of cobinamide as a post H2S exposure antidote. (Supported by NIH grant NS089487)

2121 Effect of Lower and Higher Exposure Duration on Lewisite-Induced Corneal Injury

D. Kumar[2], N. Tewari-Singh[2], D. G. Goswami[2], R. Kant[2], C. R. Croutch[1], R. P. Casillas[1], D. A. Ammar[2], J. M. Petrash[2] and R. Agarwal[2]. *[1]MRIGlobal, Kansas City, MO and [2]Department of Ophthalmology, University of Colorado Denver, Aurora, CO; [3]Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Lewisite (LEW), a potent arsenical vesicating chemical warfare agent, poses a continuous risk of accidental exposure in addition to its feared use as a terrorist weapon. The eye tissue is particularly more sensitive to LEW and exposure to even small quantities can cause devastating corneal lesions. However, detailed pathogenesis of corneal injury and related mechanisms from LEW exposure are not available. Also, to identify and screen therapies to treat ocular injury from LEW, useful clinical, histopathological and molecular endpoints are essential. Hence, using a more consistent and efficient exposure system, LEW vapor (0.2 mg/L)-induced progression of corneal injury was evaluated in New-Zealand white rabbits. Right eye of each rabbit was exposed for 2.5, 5.0, 7.5 or 10.0 min to LEW vapor, and corneal injury was assessed at 3 and 28 day post-exposure. Based on this dose-response study, acute and chronic effects of LEW exposure were further examined at 2.5 and 7.5 min exposure times for up to 56 day post-exposure in the present study. LEW vapor exposure for 2.5 and 7.5 min led to an increase in corneal thickness at all the study-time points. LEW-induced epithelial degradation was observed starting at 3 day post-exposure, which was maximal at 7 day post-exposure followed by recovery at later time points. LEW exposure also led to an increase in the number of blood vessels and inflammatory cells, and a decrease in keratocytes at 7 day post-exposure at both the exposure times; however, at lower exposure of 2.5 min, reversal of these toxic effects was evident thereafter. A significant increase in epithelial-stromal separation was observed only in the corneas exposed to 7.5 min starting at 7 day post-exposure. LEW exposure also caused an increase in the expression levels of COX-2, VEGF and MMP-9 at all the study time points indicating their role in the observed inflammation, vesication and neovascularization. The outcomes here provide valuable LEW-induced corneal injury endpoints at both lower and higher exposure duration in a relevant model system. This could help in additional studies to delineate pathways involved in corneal injury and healing from LEW exposure, and to identify and screen therapies against LEW-induced corneal injury.

2122 Screening Therapies to Counteract Chlorpyrifos Toxicity in Zebrafish

A. Govindarajan, J. J. Stegeman and J. Goldstone. *Biology, Woods Hole Oceanographic Institution, Woods Hole, MA.*

The organothiophosphate pesticide chlorpyrifos (CPF) is a high priority chemical threat agent that acts as a neurotoxicant after being metabolized by cytochrome P450 oxidases (CYPs) to the oxon form. This oxon form is active as an acetylcholinesterase inhibitor. Using larval zebrafish, we have developed a behavioral screen for drugs that to reduce the individual effects of thion pesticide exposure on these sensitive stages. Developmental chlorpyrifos exposure in zebrafish leads to altered locomotor and dark phototaxis behaviors at CPF doses that do not elicit acute cholinergic toxicity. CPF dose-dependently inhibits the dark phototaxis behavior of zebrafish older than 4dpf, but does not affect younger larvae. Drugs were screened as CPF counteragents by evaluating the restoration of dark phototaxis behavior following exposure to CPF and the prospective counteragent. Several clinically approved drugs were found to be neuroprotectants at doses similar to those used in the CPF experiments, notably verapamil (functioning as a CYP inhibitor) and menadione (a POR inhibitor). Using a combination of molecular modeling and isoform-specific CYP knockdown in zebrafish we have identified several CYP2 enzymes involved in CPF-oxon formation, and also found that CYP3A65 is critical for CFP detoxification in zebrafish. These studies provide the 'mouth' of a drug-development pipeline focused on organophosphate threat agents. (NIH CounterACT R21NS084897)

2123 Debridement of Sulfur Mustard Vapor-Induced Partial Thickness Injuries in Hairless Guinea Pig and Minipig

T. Culley[2], C. R. Croutch[2], F. Reid[2], W. Sosna[2], J. Roseman[2] and D. Barillo[1]. *[1]Disaster Response/Critical Care Consultants LLC, Mt Pleasant, SC and [2]Medical Countermeasures, MRIGlobal, Kansas City, MO.*

Rationale: Sulfur mustard rapidly penetrates skin causing a delayed injury response. We investigated efficacy of two mechanical debridement methods, wet dressings with Sulfamylon® soaks and laser, for superficial dermal and moderate deep dermal sulfur mustard burns in hairless guinea pigs (HGP) and Gottingen minipigs (MP). Methods: Twelve HGP and 6 MP had Sulfamylon®-soaked dressings applied daily for up to 10 days on sulfur mustard vapor cap injuries beginning 48 h after exposure. Optimal Sulfamylon® debridement was defined as observation of healthy granulation tissue or completion of 10 days. Twenty-six HGP (laser depth 75-600 µm) and 10 MP (laser depth 10-260 µm) used the Sciton 2940 nm Er:YAG Contour TRL Resurfacing Laser at 48 h after exposure to ablate mustard vapor cap lesions (penetration depth as evidence of coagulative necrosis and not cellular debris depth). Digital images, modified Draize score, granulation tissue, and histopathology was assessed. Results: HGP's with both injury depths became hypothermic with the wet dressings and pustules developed after 5-7 days of soaks. The hypothermia was resolved by external warming. MPs showed no granulation in superficial dermal wounds debrided with Sulfamylon® after ten days and slight granulation observed in deep dermal wounds after five days of debridement. With laser, the entire wound could be ablated and the targeted depth of ablation was reached for both depths of injury in both species. However, in both the HGP and MP DD wounds, the laser depths initially targeted did not completely remove the sulfur mustard injury. Conclusion: Both wet soaks and laser debridement are effective. Recommended laser settings and depth of ablation in HGP are 500 µm for superficial 1000 µm for deep dermal lesions and MP are 200 µm and 400 µm respectively. With Sulfamylon® for superficial and moderate dermal sulfur mustard vapor injuries, a 5 day application is recommended in MP and 4 day application recommended for HGP. Hypothermia limits use of HGP models for wet dressings.

2124 Dorsal Dermal Partial Thickness Injury in Göttingen Minipigs Induced with Sulfur Mustard Vapor

C. R. Croutch[2], D. Barillo[1], F. Reid[2], T. Culley[2], W. Sosna[2] and J. Roseman[2]. *[1]Disaster Response/Critical Care Consultants LLC, Mt Pleasant, SC and [2]Medical Countermeasures, MRIGlobal, Kansas City, MO.*

Scope: Sulfur mustard vapor (SM) penetrates the skin quickly inducing a delayed injury. Injuries are described as erythema to third degree or deeper injuries with bullous blister formation .The current treatment for SM exposure is supportive care. Recent events indicate the need for reliable animal models to test therapeutics or medical devices designed to improve healing, protect the lesion, and/or reduce/eliminate the injury seen after a sulfur mustard vapor exposure. Methods: This study developed two depths of sulfur mustard dermal injury (superficial dermal and moderate dermal) in eight Gottingen minipigs (MP, Marshall Farms) weighing ~11 kg and ~5 months of age. Each of eight sites (4 sites per side of the dorsal midline) per MP was exposed to SM vapor caps and varied exposure times (5-120 min). Control and each wound site per MP were evaluated (Pre-SM 0, 24- and 48-h post-exposure) for modified Draize score, transepidermal water loss (TEWL), ultrasound and digital images, ballistometry, and colorimetry. At 48 h post-exposure, MPs were euthanized to collect skin punch biopsies for histopathology analysis. Results: A time dependent dose-response of increasing depth of injury and wound severity by 48 h post-injury was demonstrated across exposure times. Erythema and necrosis scores, biomechanical measurements, and histopathology assessments reflected these results when comparing sham versus increasing exposure times ($p \leq 0.05$). Microscopic changes in the MP skin were consistent with human skin exposure (microvesicles, ulceration, and degeneration/necrosis) using the saturated SM vapor cap exposure times of 10-120 min on the MP dorsum. Optimum exposure times were 30 minutes to produce a superficial dermal wound (~40 µ depth) and 90 minutes (~180 µ depth) for a deep dermal wound. Conclusion: Optimum vapor exposure times were determined for a reproducible and reliable porcine model of superficial and deep dermal sulfur mustard vapor injury for use in future experiments of civilian and military interest.

2125 Attenuation of Sulfur Mustard Induced Lung Injury Using a Novel Catalytic Antioxidant

C. McElroy[1,2], E. Min[1], J. Huang[1], J. Loader[2], T. Hendry-Hofer[2], R. Garlick[2], J. Rioux[2], L. Veress[2], R. Smith[2], C. Osborne[2], D. Anderson[3], W. Holmes[3], D. Paradiso[3], C. White[2] and B. Day[1]. *[1]National Jewish Health, Denver, CO; [2]University of Colorado, Aurora, CO and [3]USAMRICD, Aberdeen, MD.*

Sulfur mustard (SM) is a particularly toxic chemical weapon due to its potent alkylating properties. Multiple models of SM toxicity have demonstrated the involvement oxidative stress in the damaging effects of SM. It was hypothesized that alleviating oxidative stress following exposure to SM with an antioxidant intervention could reduce the tissue damage. To address this hypothesis, a broad spectrum catalytic antioxidant (AEOL 10150) was selected for evaluation as a potential medical countermeasure against SM using a lethal inhalation exposure model. For the SM injury model, rats were exposed to 1.4mg/kg of SM or vehicle (EtOH) by means of a vapor generator system. Subcutaneous administration of catalytic antioxidant or PBS was initiated 1 hour post-exposure and repeated every 4 hours until the end of the study. Animals were monitored for oxygen saturation, heart rate, and clinical scores, prior to euthanasia. Upon euthanasia, lungs were either fixed for cast scoring or lavaged and then frozen. Lavage fluid and blood were collected for biochemical analysis. At 48 hours post-exposure, there was a 52% increase in survival, a 10% increase in oxygen saturation, a 22% increase in heart rate, and a 57% reduction in clinical score in animals receiving the antioxidant treatment compared to the saline treated group. At 24 hours post-exposure, antioxidant treatment reduced development of the occlusive airway casts associated with SM exposure by 57% in main airways and by 69% in total scored airways. Circulating leukocyte counts in antioxidant treated animals at 24 hours showed a 50% improvement over the SM/PBS group. Antioxidant treated groups also exhibited reduced markers of oxidative stress and inflammatory cytokines at 24 hours. Decreased BALF TGFβ1 levels indicate that rats receiving the antioxidant were also protected from a pro-fibrotic response to SM. This data indicates a pivotal role for oxidative stress in the mechanism of SM-induced lung toxicity and supports the effectiveness of using a catalytic antioxidant as a medical countermeasure for SM inhalation. Support provided by NIH-CounterACT grant U54ESO15678.

2126 Comparative Effects of Silibinin and AEOL 10150 in Reversing Skin Injury from Topical Nitrogen Mustard Exposure

A. K. Jain[4], D. Kumar[4], N. Tewari-Singh[4], R. Kant[4], D. G. Goswami[4], C. Agarwal[4], D. J. Orlicky[4], C. W. White[4], B. J. Day[1] and R. Agarwal[4]. *[1]National Jewish Health, Denver, CO; [2]Pathology, University of Colorado Denver, Aurora, CO; [3]Pediatrics, University of Colorado Denver, Aurora, CO and [4]Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Currently, there are no effective therapies available to treat primary vesicating agents, sulfur mustard (SM) and nitrogen mustard (NM)-induced blistering and incapacitating skin injuries. Our recent studies have shown the therapeutic potential of silibinin and AEOL 10150 in attenuating NM (3.2 mg/mouse)-induced acute skin injury in SKH-1 hairless mice. In this study, we further examined the efficacy of both these agents in diminishing the skin injury caused by sub-lethal (1.6 mg/mouse) dose of NM. SKH-1 hairless mice were exposed topically to a single dose of NM and clinical observations as well as skin injury biomarker studies were carried out up to 63 days post-NM exposure. For efficacy studies, dorsal skin of mice was treated 1 h after NM exposure with either topical silibinin (2 mg/mouse every 24 h) for 1 week or with subcutaneous injection of AEOL 10150 (10 mg/kg/mouse every 6 h) for 2 weeks. NM exposure reduced mice survival to 50% following 2 weeks of its exposure; however, silibinin and AEOL 10150 treatments caused 83% and 67% survival, respectively. Silibinin and AEOL 10150 treatments also significantly decreased NM-induced edema, erythema, wounding and micro-blistering in mice. Evaluation of the skin damage biomarkers for efficacy studies after treatments was carried out at 1, 3 and 7 days post-NM exposure, based on their optimal levels. Silibinin and AEOL 10150 treatments showed 77% and 62% reduction in NM-induced microvesication, respectively, and 78% and 53% reduction in NM-induced apoptotic cell death, respectively. At 7 day post NM-exposure, silibinin and AEOL 10150 treatments caused a complete or 89% reversal, respectively, in myeloperoxidase, and a significant reversal in NM-induced H2A.X phosphorylation at 1 day post NM-exposure. These findings indicate attenuation of NM-induced skin injuries by silibinin and AEOL 10150 by possibly targeting signaling pathways associated with DNA damage, inflammation, and vesication. Together, these results support further efficacy studies with these agents in vesicants-induced skin injury models.

2127 Analytical Method Developments for the Cyanide Antidote CAX1 for Solubility, Pharmacokinetics and Blood-Brain Barrier Penetration Characterization

L. Kiss, C.-E. Chou, B. A. Mendenhall, S. R. Crews, V. Coronado, J. Lowry, X. Dong, R. Jacob Roy, M. Kaur, M. N. Alam, M. S. Hossain, D. E. Thompson and I. Petrikovics. *Department of Chemistry, Sam Houston State University, Huntsville, TX.*

CAX1 is a highly lipophilic sulfur donor type cyanide antidote molecule. Various surfactants, co-solvents, and lipid based encapsulation techniques, such as micelles have been employed to make the molecule suitable for intramuscular administration. Since cyanide is a rapid acting toxic agent, the rapid absorption and brain penetration are basic requirements for the efficient cyanide antidote candidates. The *in vivo* behavior, such as absorption, distribution, and elimination have been determined on mice and rat models. The blood-brain penetration has been modelled on a triple-culture cellular model, and the *in vitro* Parallel Artificial Membrane Permeability Assay (PAMPA). This study focuses on the analytical method developments and validation for the variously formulated test sulfur donor molecule (CAX1): GC-MS- Solid Phase Micro Extraction (SPME) method was employed for measuring the molecule in the brain; HPLC-UV detection was employed for the formulation/solubility characterization, the absorption kinetics studies (blood concentration) and the cell model/PAMPA methods. For the brain study the calibration curve was prepared in the range 0.06 - 60 µg CAX1/ml of brain homogenizate and analyzed by GC-MS. The R2=0.996 and the limit of detection was 0.28µg/ml. The calibration curve for the HPLC-UV detection was prepared in the range of 0.1-100µg/ml in blood for the absorption kinetics or Ringer-Hepes buffer for the cell and PAMPA. The R2= 0.999 and the detection limit for the blood was 0.79µg/ml. The R2=0.999 and the detection limit for the Ringer-Hepes was 1.4 µg/ml. Supported: NIH CounterAct Program (AOD12060-001-00000/A120-B.P2012-01); Robert Welch Foundation at SHSU, Huntsville, TX

2128 Novel Radiation and Chemical Mitigators and Anticancer Drugs

R. Schiestl[2], Y. Rivina[1] and M. Davoren[2]. *[1]Stanford, Palo Alto, CA and [2]Pathology and Environmental Health Sciences, UCLA, Los Angeles, CA.* Sponsor: R. Schiestl.

The possibility of a radiation disaster from a nuclear detonation or accident has existed for over 50 years. The recent Fukushima accident was yet another reminder that there remains a dire need to develop novel therapies against radiation-induced toxicities. Here we report on the development of two novel radiation countermeasure therapies: CJ010: 3-Thiophenecarboxamide, 2-[[2-[benzoyl(2-furanylmethyl)amino]acetyl]amino]-4,5-dimethyl- and Yel002: 2(1H)-Quinolinone, 3-[3-[(2-furanylmethyl)amino]-6-methylimidazo[1,2-a]pyridin-2-yl]-7-methoxy-. These small, biologically active, drug-like molecules were uncovered in the DEL high throughput assay reducing radiation-induced cyto- and geno-toxicity in yeast. Radiation-modulating activity was further confirmed in yeast plate-based DEL Assay: addition of either CJ010 or Yel002 to irradiated cultures reduced cell death and genomic instability. Further, the compounds increases survival to 75% *in vivo* following an LD100/30 dose of ionizing radiation (IR) with the first therapeutic injection administered 24 hours post exposure followed by injections at 48,72,96, and 120 hours. Additionally, treatment with Yel001 an analog of CJ010 and Yel002 compounds reduces radiation-induced leukemia from 90% to to 50% and 40% respectively. Treatment with either Yel001 or Yel002 reduced spontaneous leukemia rate from 10% to 0%. Treatment with Yel002 following IR accelerates the recovery of the hematopoietic cells after sub-lethal exposures. In addition, treatment with Yel002 reduces EMS, MMS, UV, radioactive iodine, cigarette smoke extract as well as nitrogen mustard induced toxicity as well as genotoxicity showing a broad application spectrum. It also prolongs live of cells in a senescence assay. In addition Atm deficient mice live 16 weeks longer with weekly injection of Yel002 which is about 12 years in human life expectancy. In addition, Yel002 complements a zebrafish model of Diamond Blackfan Anemia. It works in yeast, CHO cells, different human cells, mice and zebrafish. Toxicity has not been observed in neither *in vitro* or *in vivo* administrations. Overall, Yel compounds have much potential as stockpile therapies for radiation-induced lethality and cancer: they are highly effective when administered up to 24hours post exposure, they reduce radiation-induce sequelae such as leukemia, and appear to have an acceptable toxicity profile.

2129 Multi System Injury Following Whole Body Exposure to Sulfur Mustard and Potential Treatments

S. Dachir, I. Rabinovitz, G. Yacov, M. Cohen, L. Cohen, H. Gutman, R. Gez, S. Chapman, V. Horwitz and T. Kadar. *Pharmacology, The Hebrew University, Jerusalem, Israel.*

Whole body exposure to sulfur mustard (HD) may be devastating resulting in multi system injuries. HD was used in many conflicts around the world and it is still a major threat for both, army troops and civilians. The aim of this study was to demonstrate the effects of HD on the eyes, skin and the respiratory system, in a most realistic scenario, using whole body exposure to HD vapor. Rats were exposed to HD vapor (LCt10-30) for duration of 10 min. Air containing HD flowed through the chamber in a constant current and the concentration was continuously monitored using FTIR. Clinical evaluation including clinical severity score and blood counts and histological evaluation were performed up to four weeks post-exposure. The potential of steroids treatment to ameliorate the HD effects was tested by betamethasone administration starting five minutes following exposure up to two weeks. Rats developed typical HD intoxication symptoms following a latency period of several hours demonstrating at first swollen and erythematic nose. This was followed by excessive rhinorrhea, eye closure, lacrimation, ronchi and wheezing, breathing difficulties, weight loss and leukopenia. Histological evaluation revealed a long lasting damage to the trachea, lungs and eyes. Treatment with steroids significantly improved clinical signs during the first week post-exposure. Later, a gradual improvement was observed in all animals. This model, of whole body exposure to HD, was found to closely mimic the deleterious effects of HD on the respiratory system and on the eyes, as was described in human victims during WWI and the Iran-Iraq war. Preliminary results indicate that steroids ameliorate part of HD effects, yet, the treatment was not sufficient to overcome the long term damages. Further research is needed to test additional combinations.

2130 A Multidisciplinary Approach for Inhalation Phosphine Poisoning

B. Wong, R. Lewandowski, H. Hoard-Fruchey, M. Martens, M. W. Perkins, T. Hamilton, J. Devorak, J. Simons, C. Rothwell, K. P. Sherman, A. M. Rodriguez, J. Tressler and A. M. Sciuto. *USAMRICD, Gunpowder, MD.*

Phosphine gas (PH3) is an extensively used reactive intermediate in the chemical industry and also a rodenticide and fumigant. National concern over phosphine's potential as a terrorist threat has increased because of its widespread use in pest control, ease of retail access, and absence of comprehensive treatment strategies. Although the exact mechanism of PH3 toxicity is poorly understood, it is thought to inhibit mitochondrial cytochromes while also causing a host of target organ and systemic effects, including oxidative stress, cardio-pulmonary toxicity, and overall metabolic disturbance. An improved understanding of the mechanisms of PH3 toxicity is central to evaluating potential therapeutic compounds and requires a multidisciplinary approach. A custom inhalation gas exposure system was designed for the whole-body exposure of conscious male rats to phosphine and coupled with real-time physiological monitoring. After PH3 exposure at concentration-time products ranging from 16500 to 21250 ppm×min, rats were euthanized at 1, 3, 6 and 24 hr post-exposure. The heart, brain, lungs, liver, kidneys, and blood were removed and analyzed using various toxicological, biochemical, genomic, and histopathologic methods to assess target organ and systemic effects. PH3 exposure induces real-time changes in pulmonary and cardiac function. ECG data from telemetry-implanted rats matched data from case studies of human PH3 exposure. Histopathological examination indicated little to no observable pathologic changes in the lung or heart, but transmission electron microscopy indicated ultrastructural damage to the cardiac tissue and mitochondria. Biochemical and genomic analysis of various tissues indicated alterations in metabolic, immune, and inflammatory cellular processes, as well as reductions in mitochondrial function. Our data suggest that PH3-induced death is due to acute cardio-pulmonary crisis and that concentration and time are not the only determinants of dose-dependent lethality. The elucidation of many aspects of PH3 poisoning has been achieved with our multidisciplinary approach, and continued use of this flexible approach will permit more effective identification of therapeutic windows and development of rational medical countermeasures and countermeasure strategies.

2131 Comparative Pulmonary Toxicity of Inhaled Metalworking Fluids in Rats and Mice

K. R. Ryan[1], M. Toraason[4], M. Cesta[1], R. Herbert[1], A. Brix[2], M. Cora[1], K. Janardhan[3], K. Witt[1], G. Kissling[1] and D. L. Morgan[1]. [1]*Division of the National Toxicology Program, NIEHS, Research Triangle Park, NC;* [2]*EPL Inc, Research Triangle Park, NC;* [3]*ILS Inc, Research Triangle Park, NC and* [4]*NIOSH, Cincinnati, OH.*

Metalworking fluids (MWFs) are complex formulations designed for effective machining operations. Adverse health effects (respiratory symptoms, dermatitis, cancer) have been reported in exposed workers. While known carcinogens have been removed from newer MWFs, recent studies in experimental animals indicate a continued health risk. This study examines the hypothesis that unrecognized health hazards exist in marketed MWFs presumed safe based on hazard assessments of individual ingredients. Subchronic inhalation studies were designed to characterize and compare the potential toxicity of four unique MWFs: Trim VX, Cimstar 3800, Trim SC210, and Syntilo 1023. These MWFs were devoid of potentially toxic contaminants present in previously used MWFs. Male and female Wistar Han or Fischer 344N/Tac rats and B6C3F1/N mice were exposed to 0, 25, 50, 100, 200, or 400 mg/m3 of MWF for 13-weeks, after which survival, body and organ weights, hematology and clinical chemistry, histopathology, and genotoxicity were assessed. Survival was not affected and toxicity was primarily limited to the respiratory tract of rats and mice. Toxicity was similar in rats and mice and gender differences were not observed. Increased lung weights (rats: 15-20%; mice: 12-50%) were associated with significant histological changes in the lung following exposure to all four MWFs. Pulmonary fibrosis was the most significant lesion caused by MWF exposure; concentration-related increases in severity and a statistically significant increase in the incidence of fibrosis were present only in rats and mice exposed to Trim VX. All four MWFs also caused lesions in the nasal cavity and larynx of rats and mice. Differences in toxicity between the MWFs were attributed to differences in chemical components (e.g., % water, biocides, hexane-extractable components, oil). These data confirm that newer MWFs have the potential to cause respiratory toxicity in workers repeatedly exposed via inhalation.

2132 Acrolein Toxicity on Larynx: Damage on Vocal Fold Epithelial Barrier Function

X. Liu, W. Zheng and M. P. Sivasankar. *Department of Speech, Language, and Hearing Sciences, Purdue University, West Lafayette, IN; School of Health Sciences, Purdue University, West Lafayette, IN.*

Acrolein is formed by combustion of fossil fuels, woods, plastics, and animal fats and also exists in tobacco smoke; chemically it is highly reactive. The vocal folds play an essential role in respiration and voice production and are at risk of acrolein exposure from inhaling polluted air. The outermost surface of vocal folds consists of epithelial cells with tight junctions serving the purpose to protect the underlying connective and muscle tissues. Our recent data suggest that acrolein has an acute toxic effect on ion transport across the vocal fold epithelial barrier. However, its influence on the protective function of the epithelial barrier has never been investigated. This study was designed to investigate the barrier integrity of vocal fold epithelia following acrolein exposure *in vitro*. The barrier integrity was evaluated for transepithelial resistance (TEER), sodium fluorescein (NaFl) permeability, and the tight junctional protein expression. Freshly isolated vocal fold epithelia from porcine larynges were exposed to acrolein (50-1300 μM) or Hanks' Balanced Salt Solution (HBSS) as controls for 3 hr. Cell viability studies using MTT assay revealed that the cell viability became significantly reduced by 27.2% when acrolein concentration reached 500 μM compared with controls ($p \leq 0.001$). Incubating vocal folds with 100 μM acrolein for 3 hr resulted in 11.35% reduction of TEER ($p<0.05$). NaFl assay further indicated a 130.5% increase in barrier permeability ($p<0.05$) following treatment with 100 μM acrolein compared with controls. The qPCR analyses showed that exposure with 100 μM acrolein for 3 hr did not cause any significant changes in expression of mRNAs encoding the tight junctional protein occludin and claudin3 ($p=0.235$; $p=0.58$); though the mRNA levels revealed the increasing trend by 102.5% and 21.6%. These findings suggest that acrolein exposure damages the vocal fold epithelial integrity and compromises its protective barrier function. Reduced TEER and increased permeability seem unlikely to be caused by altered gene expression of tight junction proteins. This study has a significant public health importance, because it has laid the groundwork for further mechanistic investigation of acrolein toxicity on larynx structure and function that are essential to people's daily voice production. (Supported by NIH/NIDCD R01DC011759).

2133 Health Guidance to Short-Term Inhalation Exposures Revisited

J. S. Snyder and E. Demchuk. *Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, Atlanta, GA.* Sponsor: P. Ruiz.

Public health responses to short-term inhalation exposures are often based on Acute Exposure Guideline Levels (AEGLs). AEGLs are threshold concentrations above which adverse health effects may take place. For each chemical, AEGLs may be derived for as many as five exposure durations. Extrapolation across durations is carried out using the toxic load model, by which Cn x t = k, where C and t are exposure concentration and duration, and n and k are chemical-specific constants. When empirical n is unknown, lower and upper statistical bounds on n are used for short-to-long and long-to-short term extrapolations, respectively. For AEGLs to be considered sufficiently health-protective, the bounds should embrace 90% of all empirical n-values. Recently, it was shown that bounds of 1.0 and 3.0, historically used in AEGL development, cover only 70% of known n-values, whereas the 90% statistical bounds correspond to n-values of 0.7 and 3.7. Using these revised health-protective bounds, new evidence-based public health guidance indicators were developed. For example, an 8-hour life-threatening threshold concentration for nitrogen mustard-1, which the AEGL Committee extrapolated from a 20 minute study, was reduced 4-fold following this revision of default n-values. Similarly, other thresholds were recalculated. These new proposed health guidance values are designed to provide a greater, science-based degree of protection for the general public (including infants, children, the elderly, and sensitive subpopulations, such as asthmatics) than previous unrevised n-values.

2134 Volatile Benzene Exposure Induces Inflammation, Insulin Resistance and Platelet Activation

W. Abplanalp[2], X. Li[2], J. McCracken[2], P. Haberzettl[2], D. Conklin[2], S. Srivastava[2], A. Bhatnagar[2] and T. O'Toole[2]. [1]*Diabetes and Obesity Center, University of Louisville, Louisville, KY and* [2]*Physiology & Biophysics, University of Louisville, Louisville, KY.* Sponsor: T. O'Toole.

Benzene is a ubiquitous volatile pollutant and is generated by petroleum, plastics, glue and shoe industries, automobile exhaust, wildfires and cigarette smoking. Consequently, it can be found in most air samples as was reported by the National Human Exposure Assessment Survey. It has been observed that benzene metabolism by hepatic CYP450 2E1 generates reactive oxygen species (ROS), which may cause inflammation, insulin resistance (IR) and endothelial dysfunction. The rapidly emerging global epidemic of human obesity, diabetes and cardiovascular disease suggests that an environmental component is likely contributing. We hypothesized that benzene exposure may enhance measures of IR and larger cardiovascular disorders. Male C57BL/6 mice were exposed to HEPA-filtered air or volatile benzene (50 ppm) for 6h/d for 14 days. Fasting plasma glucose levels (FPG), fasting insulin and HOMA-IR were increased after 14 days of benzene exposure, suggesting a rapid development of systemic IR. Supporting this was decreased insulin sensitivity in liver and skeletal muscle as measured by insulin-stimulated phosphorylation of Akt. Glutathione levels decreased in the liver of benzene exposed mice, indicating increased ROS generation. Furthermore, NF-kB p65 phosphorylation, a marker of inflammation, was increased in liver and skeletal muscle in benzene-exposed animals. Platelet leukocyte aggregates (PLAggs), and index of thrombotic potential, demonstrated a 1.7 fold increase in exposed mice compared to air controls. Treatment with the anti-oxidant 4-Hydroxy-TEMPO reversed the benzene-induced increase in FPG and PLAgg formation. These data suggest that benzene exposure induces ROS production, inflammation, insulin resistance and platelet activation. Moreover, these findings support our hypothesis that benzene exposure may be contributing to the diabetes and cardiovascular disease epidemics being observed globally.

2135 Systemic Metabolic Impairment and Lung Injury Following Acrolein Inhalation

U. P. Kodavanti[4], M. C. Schladweiler[4], A. D. Ledbetter[4], M. McGee[1], J. E. Richards[4], D. Andrews[4], D. B. Miller[3], A. Henriquez[3] and S. Snow[2]. [1]*ORISE/US EPA, Research Triangle Park, NC;* [2]*ORISE/US EPA, Research Triangle Park, NC;* [3]*UNC, Chapel Hill, NC and* [4]*US EPA, Research Triangle Park, NC.*

A single ozone exposure causes pulmonary injury and systemic metabolic alterations through neuronal and hypothalamic-pituitary-adrenal axis activation. Metabolically impaired Goto Kakizaki (GK) rats with non-obese type-2 diabetes are more sensitive to ozone induced changes than healthy rats. In order to understand how acrolein, an upper airway sensory irritant, might produce differential systemic and pulmonary responses compared to the effects produced by ozone, a deep lung irritant, we exposed healthy male Wistar and GK rats (18 week-old) to air or acrolein. In the first experiment, rats were exposed to air or 2ppm acrolein, 4h/day for 2 days and in the second experiment, rats were exposed to air or 3ppm acrolein x 4h/day for 1 day. Pulmonary ventilatory parameters, injury, inflammation as well as systemic changes in glucose tolerance, circulating lipids, metabolic markers and stress hormones were assessed. Baseline blood glucose, stress hormones, bronchoalveolar lavage fluid (BALF) LDH activity and lung protein leakage were higher, but insulin levels were lower in GK relative to Wistar rats. Acrolein at 2ppm concentration did not change systemic or pulmonary injury/inflammation outcomes. However, 3ppm acrolein exacerbated hyperglycemia and glucose intolerance in GK rats. Acrolein exposure also increased serum triglycerides but only in GK rats. Acrolein exposure at 3 ppm did not produce pulmonary protein leakage but increased γ-glutamyl transferase activity in lavage fluid in both strains, indicating airway cell injury. Acrolein (3ppm) caused neutrophilic inflammation but only in Wistar rats. Interestingly, acrolein also induced eosinophilic lung inflammation in both strains (Wistar>GK). Collectively, these data suggest that although lung inflammatory effects of acrolein might be suppressed in diabetic rats, these rats will have greater metabolic impairment from acrolein exposure. Further, acrolein, like ozone, can produce systemic metabolic alterations in diabetic rats. (This abstract does not reflect US EPA Policy).

2136 Acute Inhalation Toxicity of Oleoresin-Capsicum-Containing Smoke Used for Crowd-Control Applications

B. J. Varsho, J. Kapeles, E. Delacruz and G. L. DeGeorge. *MB Research Labs, Spinnerstown, PA.*

Oleoresin Capsicum (OC) is an oily resin found in cayenne and other varieties of peppers and is used in law enforcement as a debilitating irritant and inflammatory agent. Although traditionally delivered as a liquid aerosol ("Pepper Spray"), combusted OC carried in smoke from pyrotechnic devices has also proven effective. The short-term inhalation toxicity of OC-containing smoke was assessed using whole-body inhalation exposure methodology. Five male and five female Crl:SD(CD) rats were exposed at a total smoke concentration of 19.4 mg/l for five minutes. Particle size of the smoke was determined using cascade impaction; the mass median aerodynamic diameter (MMAD) was 1.9 µm. The concentration of the total smoke was determined by gravimetric technique, and the corresponding OC concentration in air was calculated based on the fraction of the combusted product containing OC. Animals were observed throughout exposure and daily for 14 days afterward. Body weights were collected on Study Days 0, 7 and 14. Early in the exposure period, all rats displayed avoidant behaviors such as efforts to escape the enclosures within the exposure chamber. By the end of the exposure period, all animals were lethargic, but regained normal activity levels after removal from the chamber and being washed free of smoke residue. All rats displayed no adverse signs at three hours post-exposure. All animals gained weight during the study, and survived to the scheduled necropsy.

2137 Inhalation Cancer Risk Assessment of Hexavalent Chromium Based on Updated Mortality for Painesville Chromate Production Workers

M. Suh[6], K. S. Crump[2], L. Mittal[5], S. Hirsch[4], R. Valdes Salgado[4], C. Bartlett[4], C. Van Landingham[3], A. C. Rohr[1] and D. M. Proctor[6]. *[1]Electric Power Research Institute, Palo Alto, CA; [2]Kenny Crump, Ruston, LA; [3]Ramboll Environ, Monroe, LA; [4]SciMetrika, Durham, NC; [5]ToxStrategies, Katy, TX and [6]ToxStrategies, Mission Viejo, CA.*

The exposure-response for hexavalent chromium [Cr(VI)]-induced lung cancer among workers of the Painesville Ohio chromate production facility has been used internationally for quantitative risk assessment of environmental and occupational exposures to airborne Cr(VI). We updated the mortality of 714 Painesville workers (including 198 short-term workers) through December 2011, reconstructed exposures using two metrics, and conducted exposure-response modeling using Poisson and Cox regressions to provide quantitative lung cancer risk estimates. The average length of follow-up was 34.4 years with 24,535 person-years at risk. Lung cancer was significantly increased for the cohort (standardized mortality ratio [SMR] =186; 95% confidence interval [CI] 145 - 228), for those hired before 1959, those with > 30 years tenure, and those with cumulative exposure >1.41 mg/m3-years or highest monthly exposures > 0.26 mg/m3. Of the models assessed, the linear Cox model with unlagged cumulative exposure provided the best fit and was preferred. Using Cox regression, we were able to control for significant lung cancer risk factors including smoking and age at first exposure in the derivation of potency and unit risk estimates. Adjusting for these variables, the occupational unit risk was 0.00166 (95% CI 0.000713 - 0.00349), and the environmental unit risk was 0.00832 (95% CI 0.00359 - 0.0174), which are 20% and 15% lower, respectively, than values developed in a previous study of this cohort. Due to the use of updated mortality data and refined dose-response modeling approaches, this study provides improved information for assessing the potential cancer risk associated with occupational and environmental exposure to Cr(VI).

2138 Threshold Mode of Action Analysis of a Genotoxic Carcinogen: A Case Study with 2-Mercaptobenzothiazole (MBT)

A. D. Phelka, V. S. Bhat, J. C. English and K. D. Cox. *NSF International, Ann Arbor, MI.*

Two quantitative cancer risk assessments for MBT were conducted in 2004; the purpose of the present assessment was to incorporate new data and update the dose-response assessment using current methodology. It has been suggested that new occupational data support an association between MBT exposure and mortality or morbidity due to large intestinal tract and urinary bladder cancer, and possibly multiple myeloma. However, these occupational evaluations lack clearly defined exposure concentrations and are confounded by concurrent exposure to chemicals other than MBT, including the suspected bladder carcinogen aniline, and failed to report on critical lifestyle parameters such as smoking history and diet. Neither large intestinal tract nor bladder tumors have been seen in F344 rats or B6C3F1 mice after chronic gavage exposure to MBT, and blood tumors have been limited to F344 rat mononuclear cell leukemias. Dose-response assessment conducted for target tissue tumors in rats (kidneys, blood, or adrenal glands, pituitary gland, pancreas and preputial glands) and mice (liver) identified a range of potential points of departure. Inflammation, hyperplasia, and non-linear dose-responses in some tissues suggested a threshold mode of action (MOA) and MBT did not bind to target tissue DNA of F344 rats *in vivo* . The rodent tumor spectrum is suggestive of an epigenetic MOA and given the potential for the thiol moiety of MBT to interact with protein thiol groups, including microtubule proteins, a nongenotoxic MOA was considered plausible. However, MBT was mutagenic and clastogenic *in vitro* in eukaryotic test systems that included metabolic activation and the only *in vivo* clastogenicity data available failed to test maximally tolerated doses and was judged inadequate. In these studies, i.p. dosing of MBT or zinc MBT did not induce bone marrow micronuclei in mice. Lacking sufficient evidence to support only a threshold MOA, a life-stage adjusted oral slope factor (OSF) of 2.0 x 10 -2 mg/kg-day -1 was calculated for MBT based on benign and malignant adrenal gland tumors in male rats, and was comparable to the OSF based on liver tumors in female mice. The corresponding allowable drinking water level for MBT is 20 ppb, which is 45-fold lower than the 900 ppb level derived considering a threshold MOA.

2139 An Updated Evaluation of Reported Chrysotile Asbestos No Observed Adverse Effect Levels (NOAELs) for Lung Cancer and Mesothelioma

J. S. Pierce[2], P. S. Ruestow[2] and B. L. Finley[1]. *[1]Cardno ChemRisk, Brooklyn, NY and [2]Cardno ChemRisk, Chicago, IL.*

Chrysotile asbestos was historically used in thousands of products in the US and is still used today in many countries. Therefore, it is important to understand the exposure-response relationship between chrysotile and asbestos-related health effects. The purpose of this evaluation was to update an analysis published in 2008 in which we characterized chrysotile NOAELs for lung cancer and mesothelioma. A literature search was performed to identify all epidemiology studies that described the health experience of cohorts predominantly exposed to chrysotile (< 10% amphiboles). All studies that provided risk estimates for mesothelioma or lung cancer, stratified by at least two levels of cumulative exposure, were included in our analysis. For each study, the NOAEL was defined as the highest exposure group at and below which there was no statistically significant increased risk for lung cancer and/or mesothelioma. An overall "best estimate" NOAEL range was estimated for each disease using the geometric mean of the lower-bound and upper-bound of the range of the NOAELs derived from each study. Sixteen lung cancer NOAELs were obtained from 14 published studies. The best-estimates of the lower- and upper-bound of the NOAELs for lung cancer were 89 fibers per cubic centimeter per year (f/cc-yr) and 168 f/cc-yr, respectively. Notably, none of the six cohorts of workers primarily exposed to medium or short fiber chrysotile exhibited an increased risk of lung cancer at any cumulative exposure level. Three cohorts were identified in which pleural mesothelioma risk was stratified according to cumulative chrysotile exposure. The best-estimates of the lower- and upper-bound of the NOAELs for pleural mesothelioma were 208 f/cc-yr and 415 f/cc-yr, respectively. This updated review reinforces our previous findings that support the existence of a cumulative exposure level for chrysotile below which lung cancer and mesothelioma risks are not appreciably raised.

2140 Diacetyl Exposure and Respiratory Disease: An Evaluation of the Current Weight of Evidence

B. L. Finley[1], M. E. Glynn[2], A. Abelmann[2] and J. S. Pierce[2]. *[1]Cardno ChemRisk, Brooklyn, NY and [2]Cardno ChemRisk, Chicago, IL.*

In 2002, concerns were raised regarding a perceived association between occupational exposure to the flavoring agent diacetyl and the development of bronchiolitis obliterans (BO), a rare and lethal respiratory disease that affects the bronchiolar and alveolar regions. These concerns were based on a study of Gilster Mary-Lee (GML) microwave popcorn manufacturing workers; since that time, the GML cohort has been re-evaluated on several occasions, numerous other diacetyl-exposed cohorts have also been assessed, long-term animal inhalation studies with diacetyl have been conducted, and naturally occurring diacetyl levels in many consumer products have been characterized. This analysis presents a current weight of evidence evaluation of the potential causal relationship between diacetyl and BO. Our findings are summarized as follows: (1) subchronic inhalation studies show that animals do not develop significant bronchiolar or alveolar effects even at concentrations orders of magnitude above those measured in the workplace, (2) this is in spite of findings indicating that animal respiratory epithelium is more sensitive (than human tissue) to diacetyl effects, (3) millions of humans are exposed daily to naturally occurring diacetyl in common consumables (e.g., cigarettes and coffee) at levels far above those suggested to pose a workplace risk of BO, yet none of these products cause this disease, and (4) the initial observations in 2002 were never observed again in diacetyl-exposed cohorts, even in follow-up evaluations of the same GML workers. Given these findings, it appears that the initial conclusions regarding diacetyl and BO are inconsistent with the currently available information, as the weight of evidence is negative for a causal relationship between diacetyl and BO.

2141 Evaluation of Nitrogen Dioxide and Airway Hyper-Responsiveness

M. Seeley and J. E. Goodman. *Gradient, Cambridge, MA.*

The current 100 ppb national ambient air quality standard (1-hour, primary) for nitrogen dioxide (NO2), and EPA's determination in the recent NO2 Integrated Science Assessment that the association between short-term NO2 exposure and respiratory effects is causal, both relied partly on controlled human exposure studies of airway hyper-responsiveness (AHR). These studies evaluate NO2's effect on bronchoconstriction in response to either a non-specific (e.g., methacholine, sulfur dioxide) airway challenge or a specific (i.e., allergen) airway challenge. A meta-anal-

ysis by Goodman et al. (2010) found increased AHR at 100 ppb NO2 but no clear concentration-response relationship up to 600 ppb. Although AHR was increased in studies in which participants were exposed while at rest, it was not increased in studies involving excercise. Moreover, AHR was generally not increased in studies involving specific airway challenges or chamber exposures. Overall, results from the Goodman et al. meta-analysis do not indicate a causal relationship between NO2 and respiratory effects. In contrast, Brown (2015) also evaluated the AHR studies and hypothesized that the findings could be explained by an exercise-induced refractory period and use of forced expiratory volume in 1 second (FEV1) to evaluate AHR. We explored the plausibility of Brown's hypotheses by 1) evaluating the nature of the refractory period with respect to exercise intensity and response to other airway challenges; and 2) analyzing data from the AHR studies to determine whether those that used FEV1 to evaluate AHR were more likely to observe an NO2 effect. We found no evidence to support the hypothesis that an exercise-induced refractory period explains the lack of NO2 effect on AHR for studies in which participants were exposed while excercising. Similarly, the NO2 AHR study results do not support the hypothesis that the lack of an NO2 effect on AHR for studies in which participants were exposed while exercising, or that evaluated specific airway challenges, can be attributed to using FEV1 to evaluate AHR. Moreover, there is little evidence of a concentration-response relationship in studies that evaluated multiple NO2 concentrations in subjects at rest. Overall, controlled human exposure studies indicate that AHR is not likely to worsen under plausible exposure scenarios (i.e., in response to specific airway challenges) at NO2 concentrations below approximately 300 ppb.

PS 2142 Diesel Engine Exhaust—A New Criteria Document for OEL Setting

G. Johanson[2], P. Taxell[1], T. Santonen[1], J. Järnberg[3] and A.-K. Alexandrie[3]. *[1]Finnish Institute of Occupational Health, Helsinki, Finland; [2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and [3]Swedish Work Environment Authority, Stockholm, Sweden.*

The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) produces criteria documents intended as scientific bases for setting occupational exposure limits (OEL). The documents may be downloaded from www.nordicexpertgroup.org. A document on diesel engine exhaust (DE), summarized below, was produced in collaboration with the Dutch Expert Committee on Occupational Safety (DECOS). It was adopted by NEG and DECOS in September 2015. DE is a complex mixture of gaseous (e.g. CO2, CO, NOX, SO2, various organic compounds) and particulate (diesel exhaust particles (DEP), e.g. elemental carbon (EC), organic compounds, sulfates, nitrates compounds). New technology diesel engines (NTDE) have significantly reduced DEP emissions. Critical effects of DE are pulmonary inflammation and lung cancer. For older technology DE (TDE), inflammation was detected in human volunteers after short-term exposure and in rats after long-term exposure at 100 and 210 µg DEP/m3 (~75 and ~160 µg EC/m3), respectively. Development of lung tumors was detected in rats at 2 200 µg DEP/m3 (~1 650 EC/m3). For NTDE, inflammatory changes were detected in rats at 3.6 ppm NO2 (13 µg DEP/m3; ~3 µg EC/m3). The effect was absent at 1.0 ppm NO2 (4 µg DEP/m3; ~1 µg EC/m3). No indication of tumor development was detected. Epi studies associate occupational exposure to TDE with increased lung cancer risk. Forty five years of occupational exposure to DE at 1, 10, and 25 µg EC/m3 is estimated to result in 17, 200, and 689 extra lung cancers per 10 000 exposed workers, respectively, by the age of 80 years. Although data allowing for direct comparison are not available, the significant reduction of DEP in NTDE is expected to reduce the lung cancer risk per kWh.

PS 2143 Evaluation of Atherosclerosis as a Mode of Action for the Cardiovascular Effects of Particulate Matter

R. L. Prueitt[2], J. M. Cohen[2] and J. E. Goodman[1]. *[1]Gradient, Cambridge, MA and [2]Gradient, Seattle, WA.*

Epidemiology studies have consistently reported associations between PM2.5 exposure and cardiovascular (CV) morbidity and mortality, but the evidence for associations between PM2.5 and subclinical measures of atherosclerosis is unclear. We conducted an analysis of the experimental studies of PM2.5 and effects associated with acceleration and exacerbation of atherosclerosis and used the principles of the International Programme on Chemical Safety (IPCS) mode of action/human relevance framework to evaluate whether they support a biologically plausible, human-relevant mode of action (MoA) for the CV effects of PM2.5 reported in the epidemiology literature. We focused our analysis on outcomes related to atherosclerotic plaque development, thrombosis, and coagulation, and we examined whether these outcomes were correlated with measures of oxidative stress and systemic or pulmonary inflammation, to evaluate whether these processes are likely to be key early events for atherogenic effects of PM. We found that the effects of PM2.5 on atherosclerotic lesion development were correlated with increased oxidative stress, but not with inflammation. The effects on thrombosis and coagulation immediately after exposure appeared to be induced by direct translocation of particles from the lungs into the systemic circulation, whereas the prothrombotic effects at later time points depended on the presence of pulmonary inflammation. Although the current experimental evidence indicates that acceleration and exacerbation of atherosclerosis by PM2.5 is a biologically plausible MoA for CV effects in experimental animal models, our analysis indicates that the human relevance of the key events in the proposed MoA is unclear and not well supported by the existing data. Further studies are needed to fill several important data gaps before the human relevance of this MoA can be established.

PS 2144 Estimating Human Equivalent Exposure Concentrations for Acetaldehyde Using CFD/PBPK Modeling

R. A. Corley, A. P. Kuprat, D. R. Einstein, J. G. Teeguarden and S. Kabilan. *Pacific Northwest National Laboratory, Richland, WA.*

Inhalation exposures to acetaldehyde vapors can produce nasal toxicity and tumors in nasal tissues of rats. In this study, a published computational fluid dynamics (CFD)/physiologically based pharmacokinetic (PBPK) model for acetaldehyde (Corley et al., 2015) was modified to include acetaldehyde-induced vasodilation and a separate mucus compartment in addition to the original cell type- or region-specific epithelial and subepithelial compartments to determine site-specific tissue concentrations of acetaldehyde in rat and human nasal tissues. With these changes to the CFD/PBPK model structure, tissue metabolism rates were re-calibrated against published (Morris and Blanchard, 1992) steady-state inhalation nasal extraction studies over a concentration range from 10 to 1000 ppm acetaldehyde with evaluations of model performance at multiple steady-state and cyclic breathing flow rates. The revised CFD/PBPK model for the rat and human was then used to refine prior PBPK-only based estimates of the human equivalent exposure concentration (HEC) for continuous exposures that corresponded to target tissue doses of acetaldehyde in rats at the NOAEL of 50 ppm in the subchronic inhalation study of Dorman et al. (2008). For this comparison, we focused upon site-specific 'hot spots' for acetaldehyde dose, defined in this study as the top 2.5% of the surface area for each cell type lining the nasal airways with the greatest time- and tissue penetration depth-dependent concentrations of acetaldehyde (apparent AUC's). Using the exposure duration adjusted hot spot tissue doses of acetaldehyde in rat olfactory epithelium exposed to a NOAEL of 50 ppm, the resulting HEC's were 11.0 ppm for human olfactory epithelium and 13.6 ppm for respiratory epithelium. Funded by Vinyl Acetate Council Project No. 65907.

PS 2145 Provisional Advisory Levels (PALs) for Cyanide (HCN, KCN, NaCN)

S. Milanez[2], C. Baird[4], M. McClanahan[3] and G. Henningsen[1]. *[1]FR-ETS, Monument, CO; [2]Oak Ridge National Laboratory, Oak Ridge, TN; [3]Retired, Centers for Disease Control and Prevention, Chamblee, GA and [4]Aberdeen Proving Ground, USA CHPPM, Aberdeen, MD.*

PAL values developed for hazardous materials by the US EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-year durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance, but are intended for use at the discretion of risk managers in emergency situations when site-specific risk assessments are not available. The US EPA through its Office of Research and Development funded and managed the research described here. The PAL protocol was applied to estimate exposure limits for cyanide (CN) as a gas (HCN) and for the cyanide ion in water, originating from HCN, KCN, or NaCN. CN is present in the air, drinking water, and in many foods. HCN is used to produce acrylonitrile, which is used to make acrylic fibers, synthetic rubber, and plastics; in electroplating; and in ore concentration. Cyanide inhibits cellular respiration in mitochondria, excessive exposure causing tissue hypoxia and cell death. Acute exposure affects primarily the CNS and heart (headaches, dizziness, numbness, nausea, palpitation); chronic exposure inhibits thyroid hormone production. Human data were adequate to develop most inhalation PAL 1 and PAL 2 values, but not inhalation PAL 3 values or any oral

PALs, which were instead based on animal studies. In some cases PALs were not recommended (NR) due to insufficient data. The developed oral PALs (mg/L) for 24-hour, 30-day, 90-day, and 2-year durations are as follows: PAL 1 = 0.42, 0.21, 0.070, NR; PAL 2 = 2.8, 0.63, 0.42, NR; PAL 3 = 7.0, 6.3, 4.2, NR; the oral PAL 3 values may not be appropriate for children under certain exposure scenarios. The corresponding duration inhalation PALs (ppm) are: PAL 1 = 0.19, 0.039, 0.039, 0.039; PAL 2 = 0.87, 0.26, 0.26, 0.26; PAL 3 = 3.0, NR, NR, NR. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Thanks to F. Adeshina and H. Choudhury of the US EPA for their contributions to this work.

2146 Derivation of Health-Protective Screening Values for Chemicals in Settled Dust on Outdoor Surfaces

C. Kuhlman and S. Wnek. *Center for Toxicology and Environmental Health, LLC, Little Rock, AR.* Sponsor: C. Kuhlman.

Following chemical release emergencies, air monitoring and sampling are often performed to evaluate whether airborne chemicals are present at levels that could potentially impact human health. Questions often arise regarding exposures to chemicals that may have deposited onto outdoor surfaces. Analytical wipe sampling can identify and quantitate the levels of chemicals present on outdoor surfaces; however, health-protective screening values for chemicals on outdoor surfaces are not readily available to evaluate the sample results. In response to the World Trade Center (WTC) disaster, the United States Environmental Protection Agency's (US EPA) Contaminants of Potential Concern (COPC) Committee of the WTC Indoor Air Task Force Working Group developed indoor settled dust screening values for numerous dust-associated constituents, including inorganic metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxins. These screening values account for the incidental ingestion and dermal contact with dust residues present on indoor surfaces. While these screening values are relevant for exposures to chemicals in settled dust in an indoor setting, the exposure assumptions used to derive indoor screening levels likely overestimate potential exposures to outdoor surfaces. Using the methods outlined in the US EPA COPC WTC Guidance Document, health-protective screening values were developed for inorganic metals, PAHs, PCBs, and dioxins present in settled dust on outdoor surfaces. The use of these screening values will allow stakeholders to evaluate potential health risks associated with exposure to outdoor surfaces potentially impacted by a chemical release emergency. Furthermore, these screening values can facilitate a rapid identification of areas that may require remediation, and can be used to assess the effectiveness of subsequent cleanup efforts.

2147 Microcystin LR-Induced Reproductive Toxicity: Implications for Risk Assessment

B. Hawkins. *National Center for Environmental Assessment, US EPA, Cincinnati, OH.* Sponsor: H. El-Masri.

Historically, the liver has been considered the primary site of microcystin toxicity and microcystin-induced hepatotoxicity has been extensively studied. However, effects on the male reproductive system have also been consistently observed with purified microcystin-LR and microcystin extracts via oral exposure and i.p. injection at low doses. The effects on the male reproductive system following microcystin exposure are varied and include histopathological changes in the testes, impacts on sperm concentration, viability and motility and alterations in serum hormone levels (testosterone, LH, and FSH). Recently, microcystin-induced effects on the female reproductive system (following i.p. injection) and neurobehavioral impacts on offspring from orally exposed female rats have also been reported at low doses. In the present study, a weight of evidence (WOE) evaluation of the available microcystin-LR animal toxicity information was conducted and potential points of departure (PODs) for hepatotoxicity and reproductive toxicity were identified from all available repeat-dose oral toxicity information using Benchmark Dose (BMD) Modeling (where appropriate). A mode-of-action (MOA) evaluation of microcystin-LR (focusing on hepatotoxicity and reproductive toxicity) was also conducted. The WOE evaluation, MOA evaluation and quantitative comparison of PODs found reproductive toxicity to be a sensitive endpoint and relevant for use in human health risk assessment. The views expressed herein are those of the author only and do not necessarily reflect the views or policies of the US EPA.

2148 Reproductive and Developmental Health Impacts of Cyanotoxins: A Comparative Review

A. Saulles[2], S. Mehta[1] and B. Foos[1]. [1]*Office of Children's Heath Protection, US EPA, Washington, DC and* [2]*School of Public Health, University of Michigan, Ann Arbor, MI.* Sponsor: E. Ohanian.

Cyanotoxins, the chemicals produced from harmful algal blooms, are increasingly prevalent in both recreational and drinking water sources in the United States potentially at levels found to be unsafe to humans. Though evidence has shown these compounds to be hepatotoxic and neurotoxic, it is largely unknown if cyanotoxins also are associated with reproductive and developmental effects. This unknown health effects information is of particular concern for infants and children as they already are at an increased risk of exposure due to both their behavior as well as their higher intake rate of food and water compared to adults, in addition to *in utero* exposures. To determine if early life-stages have an increased health risk when exposed to cyanotoxins, a comparative review of relevant studies on cyanotoxin toxicities was conducted. Through our analysis, only a limited number of studies with reproductive and developmental effects were found across all cyanotoxin species. Studies using human cells, rodents and fish, examining a variety of endpoints including sperm motility, cell loss and damage, and endocrine disruption were identified. However, no human exposure or epidemiology studies have been conducted. Although some studies have identified cylindrospermopsin and microcystin as developmental and reproductive toxins, significant critical research gaps exist. Further research, especially that addressing the possibility of differential toxicity between ages and sexes, will clarify cyanotoxins' role in reproductive and developmental toxicity, and inform future risk assessment and risk management decisions that protect the health of bottle-fed infants and young children.

2149 Meta-Regression Analysis of Lung Cancer Risk and Inorganic Arsenic in Drinking Water Studies

H. Ferdosi[1], S. Lamm[1], J. Ahn[2] and J. Li[3]. [1]*Center for Epidemiology and Environmental Health, CEOH, LLC., Washington, DC;* [2]*Department of Biostatistics, Bioinformatics & Biomathematics, Georgetown University, Washington, DC and* [3]*Department of Pathology, Johns Hopkins School of Medicine, Baltimore, MD.*

High levels (~ 200 ug/L) of inorganic arsenic in drinking water are known to be a cause of human lung cancer, while the evidence at lower levels is uncertain. To examine the dose-response relationship of lung cancer risk and drinking water arsenic levels we have sought all such studies that include high exposures, low exposures, and intermediate exposures. We have used all the data from the six studies in a meta-regression analysis. The best fit on a log-log plot over the range of 1 - 1,000 ug/L is a linear-quadratic curve with a positive quadratic term and a negative linear term. Each study fits this model, as do all studies together and studies grouped by study design (ecological, non-ecological, case-control, and cohort). The analyses for the ecological studies were as informative as those for the non-ecological studies and yielded similar results. Statistically significant X-intercepts consistently found a no-increased level of risk at about 100-150 ug/L arsenic and for all studies together at 136 ug/L.

2150 Applying the "Severe Toxicity" Factor for Non-Genotoxic Carcinogenicity When Deriving PDE Values

J. W. Card, H. Fikree and L. A. Haighton. *Intertek Scientific & Regulatory Consultancy, Mississauga, ON, Canada.*

The ICH method for deriving a permitted daily exposure (PDE) value is comparable to other risk assessment methods in that the most relevant NO(A)EL is identified and then reduced with the application of numerical modifying factors (also known as safety or uncertainty factors) to relate the data to humans. A unique aspect of the PDE method is the application of a modifying factor ("F4") to account for severe toxicity, which may apply in cases of "non-genotoxic carcinogenicity, neurotoxicity, or teratogenicity". Detailed guidance is provided for using F4 values of 1, 5, or 10 for teratogenicity but is lacking for non-genotoxic carcinogenicity. Based on principles of toxicology, we applied the following rationales to define the use of the F4 factor: F4=1 if tumor type is known to be not relevant to humans (e.g., α2u-globulin mediated), tumor incidence is specific to a route that is not relevant to the PDE route of exposure (e.g., titanium dioxide), or the compound is endogenous and toxicity is not expected at low doses (e.g., hydrogen peroxide); F4=5 if tumor is found only in one test species for which historical incidence is high; and F4=10 if tumor type is rare or if tumors are found in more than one test species.

To demonstrate the application of this approach, PDEs were derived based on publicly-available information for midazolam maleate and methylphenidate hydrochloride (both negative in Ames and micronucleus assays) to comply with the new EMA guideline on manufacturing drugs in shared facilities. High-dose midazolam maleate was associated with benign and malignant liver tumors in female mice and benign thyroid follicular cell tumors in male rats. Although liver tumors are common in mice, an F4 factor of 10 was applied given that malignant tumors were observed and some tumor findings also were reported in rats. In contrast, high-dose methylphenidate hydrochloride was associated with only benign liver tumors in mice and no tumors in rats; therefore an F4 factor of 5 was used. Standardizing the use of the F4 factor in this manner will assist with derivation and justification of PDE values.

2151 Derivation of a Permitted Daily Exposure Value for Vincristine as a Carryover Contaminant in Drug Product

H. Fikree, J. W. Card and L. A. Haighton. *Pharmaceuticals & Healthcare, Intertek Scientific & Regulatory Consultancy, Mississauga, ON, Canada.*

Carryover of a drug substance or active pharmaceutical ingredient (API) into another drug product represents a potential risk for cross-contamination that can be of concern to a patient who is subsequently administered the contaminated drug product. Published guidance documents and thought papers on cross-contamination of APIs have recommended methods to establish a safe exposure limit [permitted daily exposure (PDE) value] that can be used to assist with cleaning validation efforts. The present work was performed to derive a PDE value for the cytotoxic anticancer agent, vincristine, as a potential carryover contaminant in other drug products. Based on guideline recommendations, repeat-dose toxicity, genotoxicity, carcinogenicity, and reproductive and developmental toxicity data were evaluated. Critical review and summary of the identified information indicates that the substance is genotoxic (not unexpected given the mechanism of action) and non-carcinogenic; however, reproductive and developmental toxicity data suggest that dose levels of ≥0.06 mg/kg/week affected male fertility and dose levels of ≥0.1 mg/kg/day were associated with embryotoxicity and/or teratogenicity in mice, rats, hamsters and monkeys. While consideration was given to basing the PDE value on the established exposure limit of 1.5 µg/day for genotoxic impurities, ultimately, and to address concerns about teratogenicity and reproductive toxicity, the results of a 9-week intravenous rat fertility study were used to derive the PDE value. Based on a no-observed-adverse-effect level (NOAEL) of 0.04 mg/kg/week for male fertility and the application of modifying factors of 5 (F1; extrapolation from rats to humans), 10 (F2; inter-individual variability), 10 (F3; study of 9 weeks in duration), 5 (F4; reversible effect on male fertility), and 1 (F5; use of a NOAEL), a PDE of 0.016 µg/kg body weight/week (0.0023 µg/kg/day) was derived. The derived PDE value is more conservative than the genotoxic impurity exposure limit and is considered to be acceptable with respect to safety.

2152 Establishing an Allowable Drinking Water Level for the Multifunctional Acrylate, Tripropylene Glycol Diacrylate

J. C. English, V. S. Bhat and K. D. Cox. *NSF International, Ann Arbor, MI.*

Tripropylene glycol diacrylate (TPGDA) is a bifunctional cross-linking agent with diverse applications in coatings, UV curable inks, varnishes, and sealants. In addition to dermal and inhalation routes of exposure, the general population may ingest TPGDA that is imparted to drinking water through contact from drinking water system components. An oral risk assessment was undertaken to establish allowable drinking water levels. The extremely limited oral data set for TPGDA was supplemented by several dermal repeated-exposure studies and by data for related multifunctional acrylates. Dermal administration of TPGDA to SD rats for 13-weeks was moderately irritating to the treatment site, and resulted in dose-related reduced body weights with apparent kidney involvement. In a nonstandard dermal carcinogenicity study, topical administration of TPGDA to male C3H/HeJ mice for 80 weeks resulted in systemic toxicity, including an increased incidence of kidney lesions relative to concurrent controls, as well as treatment site fibrosis but no skin tumors. The weight of evidence based on data available for TPGDA and numerous other multifunctional acrylates indicates these compounds have clastogenic activity *in vitro*, but this activity is not observed in standardized *in vivo* micronucleus assays. In non-standard rat teratology screening assays, administration of TPGDA on gestation days 6-15 did not show any evidence fetotoxicity regardless of whether the oral or dermal route was used; whereas clear evidence of maternal toxicity was observed after 250 mg/kg-day dermal and 500 mg/kg-day gavage administration. Generalized systemic toxicity from the subchronic dermal study (NOAEL of 48 mg/kg-day) was selected as the critical effect to inform an upper estimate of risk associated with the oral route of exposure, recognizing that detoxification pathways are more readily saturable with repeated dermal as compared with oral exposure. Consequently toxicity is attenuated by the oral route relative to the dermal route. The Human Equivalent NOAEL is 12 mg/kg-day and total uncertainty factor of 1000x (3x interspecies, 10x intraspecies, 3x duration, and 3x database), yields an oral RfD of 0.01 mg/kg-day. The Total Allowable Concentration in drinking water of 80 µg/L, takes into account sources of exposure other than drinking water.

2153 Derivation of Drinking Water Criteria and Oral Risk Assessment of the Antimicrobial Agent Triclosan

K. D. Cox and J. C. English. *NSF International, Ann Arbor, MI.*

Triclosan is used as an antimicrobial agent in a wide range of consumer products/applications which may result in triclosan being imparted indirectly into drinking water. Oral and dermal exposures to triclosan occur predominantly through the use of consumer products containing triclosan. The purpose of the present assessment was to incorporate current risk assessment methodology and new data to derive an updated oral RfD from which to establish drinking water criteria. As available human safety studies reported no signs of overt toxicity in over 3,000 subjects exposed to triclosan-containing products, the hazard identification for triclosan relies on laboratory animal data. In repeated dose oral studies in laboratory animals, adverse effects of triclosan were reported predominantly in the liver, kidney and blood. Hepatic tumors were reported in mice following 18-month exposure to triclosan but the available data suggest that such tumors were likely due to mouse-specific PPARα activation; additionally, no evidence of carcinogenicity was reported in long-term dietary studies in both rats and hamsters. The available *in vivo* and *in vitro* genetic toxicity studies for triclosan suggest low concern for genotoxicity. Reproductive and developmental effects following exposure to triclosan were reported at concentrations at which maternal (or parental) toxicity was typically present; however, recent studies in rats have demonstrated consistent reductions in serum T4 concentrations following exposure to triclosan. Based on liver histopathology and decreased erythroid parameters reported in mice following subchronic and chronic exposure to triclosan, a NOAELHED of 1.4 mg/kg-day was selected as the point of departure, and was determined to be adequately protective against other adverse effects considered, including thyroid disruption and renal toxicity. Using a 30x UF to account for inter- and intraspecies variability, an updated oral RfD of 0.047 mg/kg-day was determined for triclosan which represents a decrease from the prior oral RfD established by NSF International in 2003 of 0.08 mg/kg-day.

2154 Oral Risk Assessment and Derivation of Allowable Drinking Water Levels for 1-Hexanol

J. A. Brickel, K. D. Cox, B. J. Lampe and J. C. English. *NSF International, Ann Arbor, MI.*

General population exposure to 1-hexanol may occur from the natural occurrence of 1-hexanol in food as well as commercial uses as a food additive and in household products. Incidental oral exposure to 1-hexanol may also occur through its residual presence in drinking water system components. An oral risk assessment incorporating current risk assessment methodology was conducted to establish allowable drinking water levels for 1-hexanol. Due to inadequate human data, the hazard identification utilized laboratory animal data, which included no statistically significant toxic responses to 1-hexanol administered for 13-weeks in albino rats at doses up to 1127 mg/kg-day. In a 13-week oral study in Beagle dogs administered 1000 mg/kg-day of 1-hexanol in a gelatin capsule, signs of central nervous system depression, difficult respiration, testicular atrophy, decreased oogenesis, and mortality were reported. These findings were attributed to the bolus dosing method and considered of limited relevance to exposure via drinking water. At lower doses administered to dogs in the daily diet (190 mg/kg-day and 370 mg/kg-day), no systemic effects were identified but localized gastrointestinal tract irritation was reported. A critical effect was not identified for the point of departure, as no treatment related systemic effects were identified in either of the two species given 1-hexanol subchronically in the diet. The weight of evidence for 1-hexanol and similar primary aliphatic alcohols suggest a low concern for genotoxicity. Using the rat NOAEL of 1127 mg/kg-day and the US EPA default Dosimetric Adjustment Factor, a Human Equivalent NOAEL of 270 mg/kg-day was derived. Based on a total uncertainty factor of 1000x (3x interspecies, 10x intraspecies, 10x

subchronic to chronic, and 3x incomplete database), an oral RfD of 0.27 mg/kg-day was established. A relative source contribution of 20% was used to account for sources of 1-hexanol exposure other than drinking water. Therefore, the Total Allowable Concentration of 1-hexanol in drinking water is 2 mg/L.

2155 Oral Risk Assessment and Derivation of Acceptable Drinking Water Levels for Nonylphenol

N. Beetsch, V. S. Bhat and J. C. English. *NSF International, Ann Arbor, MI.*

The lack of published risk estimates for nonylphenol (NP) prompted the present assessment to identify new data since previous toxicity reviews and to derive acceptable drinking water levels for chronic NP exposure. Oral data for any NP isomer were considered (CAS#s 25154-52-3, #84852-15-3, #104-40-5), since NSF International detects 2/4-isomeric mixtures from NP-containing materials with potable water applications. These data consistently identify the kidney as the target organ in both sexes of rats, with males more sensitive at ≥15 mg/kg-d. Four standard studies assessed renal and reproductive effects, with 3 studies also the longest-term studies. Branched 4-NP was tested in two dietary 3-gen studies (Chapin et al., 1999; Tyl et al., 2006). Branched and linear 2/4-NP was tested via gavage in a 2-gen study (Nagao et al., 2001). The dietary NOAEL and LOAEL for renal lesions of 15 (life-stage range 10-25) m/k-d and 45 (range 30-90) mg/kg-d, respectively, is supported by the gavage NOAEL and LOAEL of 10 and 50 mg/kg-d, respectively. Gender and life-stage differences in sensitivity and dietary NP intake were seen. Comparatively, the weight-of-evidence (WOE) NOAEL for specific effects on reproduction or development is 15 mg/kg-d, with the most sensitive effect being decreased fetal viability. The proposed mode of action for the critical effect, renal corticomedullary mineralization in PND 21 F1 male rats, is altered renal calcium homeostasis, considered to have a threshold and potential relevance to human health. Limited toxicokinetics data (10 or 100 mg/kg-d NP via gavage or 45 mg/kg-d via diet) support a metabolic threshold in the range of observed renal responses. Most NP was metabolized in the liver, with the majority of metabolites excreted in bile as glucuronide conjugates at 10 mg/kg-d with no detectable parent. Greater proportions of parent compound are detected at ≥45 mg/kg-d. Recognizing large fluctuations in achieved dietary intakes, the point-of-departure (POD) for the RfD was the gavage NOAEL for 2/4-NP due to consistency in achieved dose and to test substance consistency with potential human exposures in drinking water. The gavage NOAEL of 10 mg/kg-d is a clear NOAEL while the dietary NOAEL of 15 mg/kg-d had a low incidence of renal lesions in both multi-gen studies. Benchmark dose modeling of alternate PODs, including accelerated vaginal opening or reduced fetal viability, was also conducted. The NOAEL approach was preferred to avoid extrapolation below the range of observation.

2156 Derivation of the Health Advisory Guidance Values for Sub-Acute Exposure of Drinking Water

T. Nishimura[3], M. Hirata-Koizumi[2], T. Yamada[2], T. Kawamura[2], A. Ono[2], A. Hirose[2] and M. Ema[1]. *[1]National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan; [2]Division of Risk Assessment, National Institute of Health Sciences, Tokyo, Japan and [3]Faculty of Pharmaceutical Sciences, Teikyo Heisei University, Tokyo, Japan.* Sponsor: A. Hirose.

The enforceable quality standards for drinking water contaminants are established in order to protect human health during lifetime exposure. After lacal bad weather or small chemical accidents, the concentration of contaminants may exceed the enforceable standards levels in transiently. If the excedding levels are not serious, a suspension of water supply should not be a realistic management option. In such case, immediate judgment is required to deal with an accident. The Health Advisories values for health effects of acute toxicity (for one day and ten days) has been developped by the US EPA. These values are considered to be very useful as the reference values for transient pollution. However, at the major drinking water suppliers in Japan, it takes around one month to completely recover the transient exceeding contaminants concentrations to normal levels. Therefore, we attempted to assess subacute reference dose (saRfD) of 19 contaminated chemicals among the Japanese drinking water quality standards, in order to establish the subacute (for one month) guidance value. The saRfD was determined by toxicity studies using laboratory animals, in principal, and a NOAEL (or a BMDL10) of a 90-day repeated dose toxicity study or a reproductive/developmental toxicity study for each chemical was used as a point of departure (POD). If no reliable information about 90-days studies was available, a NOAEL of the longer study was selected as a POD. The uncertainty factors of 100 were generally applied to the POD. As for genotoxic carcinogen, the dose corresponded to the risk of 3 x 10-5 was defined as a saRfD. Finally, the subacute advisory guidance value was set for protecting a 10-kg child consuming 1 liter of water per day, and no allocation factor from a saRfD was used. Acknowledgment: This study was supported by a Health and Labour Sciences Research Grant (H25-Kenki-Ippan-007) from the MHLW, Japan.

2157 Chemical Hazard Assessment of Guanidine Compounds by Categorical Regression of Human Case-Report Data

D. Petersen. *NCEA, US EPA, Cincinnati, OH.* Sponsor: D. Petersen.

In the development of a recent human health risk assessment of guanidine nitrate, no repeated-dose human or animal studies (subchronic or chronic duration) of guanidine nitrate were located. However, guanidine chloride has been used in the management of Lambert Eaton Myasthenic Syndrome (LEMS) in humans, and thus the appearance of side effects in treatment could be used as a surrogate for guanidine nitrate to identify toxicological effects and evaluate dose-response. Side effects were grouped by severity for the purposes of categorical regression, and the response variables were: Category 1 (no symptoms), Category 2 (mild; serum chemistry changes <10%), Category 3, (moderate; larger serum chemistry changes, salivation, sweating, parasthesias, mild GI symptoms) or Category 4 (severe; hypotension, neutropenia, unmanageable GI (vomiting and diarrhea), seizures). Twenty case reports, several with multiple patients, were analyzed for dose (in mg/kg), duration, and the response categories above. Several patients were treated with multiple doses, with varying side-effects recorded at each dose. Categorical regression (CatReg) analysis (R v 3.10) identified the lower confidence limit of an effective response concentration 10% (ERC10) of 2 mg/kg-day, based on the Cloglog model with linear dose. That value was used as a point of departure (POD) for the derivation of provisional subchronic and chronic reference doses (RfDs) for guanidine chloride and other guanidine compounds. The views expressed in this abstract are those of the authors and do not necessarily reflect the views and policies of the US EPA.

2158 Derivation of Toxicity Factors for a Set of Structurally Diverse Isocyanates

T. Bredfeldt and J.-S. Lee. *Toxicology, Texas Commission on Environmental Quality, Austin, TX.*

Isocyanates are widely used in the production of foams, fibers, coatings, adhesives, elastomers and building insulation material. There is a diverse array of isocyanates available for various commercial and industrial applications. However, toxicity factors have been derived primarily for the data-rich members of this class of chemicals, including methyl isocyanate (MIC), toluene diisocyanate (TDI), hexamethylene diisocyanate (HDI), and methylenediphenyl diisocyanate (MDI). There is significantly less data available to evaluate the toxicity of other monomeric and polymeric isocyanates. Current data indicates that members of this group of chemicals have similar modes of action that are primarily driven by the isocyanate moiety itself, which is highly reactive. Upon inhalation, isocyanates induce pulmonary and sensory irritation. Prolonged or high-concentration exposures may also cause sensitization, resulting in increased sensitivity to the isocyanate and asthma. Sensitization may occur in response to both monomeric and polymeric isocyanates. In this study, we present toxicity factor derivation for a set of monomeric and polymeric isocyanates. Using information from the data-rich isocyanate monmers and polymers, we apply various methods for toxicity factor derivation for chemicals with limited toxicity data, including read-across, relative potency, and surrogation, to arrive at a final set of toxicity factors that reflect integration and interpolation of data across structurally diverse isocyanates.

2159 Derivation of Relative Potency Factors for Toxicity Surrogates of Data-poor Chemicals

P. R. Dodmane[1], J. P. Kaiser[2], Q. J. Zhao[2] and S. C. Wesselkamper[2]. *[1]ORISE Participant, US EPA, National Center for Environmental Assessment (NCEA), Cincinnati, OH and [2]US EPA, NCEA, Cincinnati, OH.*

Quantitative human health risk assessment of chemicals with little-to-no toxicity data poses a significant challenge. Alternative computational toxicological methods can be applied to derive reference values for such chemicals based on toxicological information of corresponding surrogates, with careful consideration of relative toxicity potencies. For

cis- and trans-nonachlor and oxychlordane which have limited toxicity data, technical chlordane was previously determined as an appropriate surrogate chemical by applying a tiered surrogate approach (Wang et al., 2012). In the present analysis, the potency differences relative to the surrogate chemical were accounted for by deriving relative potency factors (RPFs) using the limited available toxicity data. An RPF can be determined for chemicals with few toxicity data that are structurally similar and have similar dose-response curves for a common endpoint. Technical chlordane and the aforementioned data-poor chemicals meet this criteria. An RPF is calculated as the ratio of the dose of an index chemical to another chemical at an iso-effective dose. An index chemical is typically the best studied chemical, and is technical chlordane in this case. A common toxic effect of hepatocyte hypertrophy in 28-day oral rat studies was identified. By applying benchmark dose (BMD) modeling to the liver data, iso-effective BMD50 values were used to derive RPFs. In female rats, the RPFs were 1.0, 1.0, 32.2, and 5.6 for technical chlordane, cis-nonachlor, trans-nonachlor, and oxychlordane, respectively. In male rats the RPFs were 1.0, 4.8, and 7.5 for technical chlordane, cis-nonachlor, and trans-nonachlor, respectively. Trans-nonachlor was determined to have the highest potency based on the RPFs. The RPFs can be used with index chemical points of departure (PODs) to calculate surrogate PODs for the data-poor chemicals. The views expressed in this abstract are those of the authors and do not necessarily reflect the views and policies of the US EPA.

PS 2160 A Case Study on the Utility of a Tiered Surrogate Approach in Quantitative Risk Assessment of 2,4,6-Trinitrophenol

L. E. Lizarraga[1] and Q. J. Zhao[2]. *[1]Oak Ridge Institute for Science and Education, Cincinnati, OH and [2]US EPA, National Center for Environmental Assessment, Cincinnati, OH.*

Conventional risk assessment methods rely extensively on toxicity data from experimental animal and epidemiologic studies to derive reference health values for environmental chemicals. Chemicals lacking *in vivo* toxicity information pose a significant challenge to regulatory agencies. One way to address this issue is to apply alternative computational approaches to characterize potential health effects and dose-response relationships. The present study combines structure-activity relationships and read-across methods to identify chemical surrogates for 2,4,6-trinitrophenol (picric acid), a chemical with a limited toxicity database. Briefly, a list of putative structural analogs with available oral reference values was compiled, using DSSTOX and ChemIDplus. Subsequently, metabolic and toxicity information for the structural analogs was compared to the target compound. Applying a weight of evidence approach, a final surrogate was selected. Six structural analogs with a >60% similarity score were identified for picric acid: 2-methyl-4,6-dinitrophenol, 2,4,6-trinitrotoluene, 2,4-dinitrophenol, 2-(1-methylpropyl)-4,6-dinitrophenol, 1,3,5-trinitrobenzene and 1,3-dinitrobenzene. Limited information on metabolism of picric acid and candidate analogs precluded the identification of metabolic surrogates. Comparison of toxicity profiles revealed similarities in adverse effects on the hematological system, spleen and testes between 1,3,5-trinitrobenzene and picric acid. Thus, 1,3,5-trinitrobenzene was selected as the most viable surrogate and methemoglobinemia in male rats exposed to chronic 1,3,5-trinitrobenzene treatment was used as a critical effect for the derivation of chronic oral reference values for picric acid. The current analysis illustrates the application of a tiered surrogate approach in identifying surrogate chemicals and assisting in quantitative risk assessment of data-poor chemicals. The views expressed in this abstract are those of the author and do not necessarily reflect the views and policies of the US EPA.

PS 2161 GenRA: Evaluating Local Validity for Read-across Prediction Using Chemical and Biological Information

I. Shah[3], J. Liu[1,2,3], R. S. Judson[3], R. S. Thomas[3] and G. Patlewicz[3]. *[1]ORISE, Oak Ridge, TN; [2]UALR, Little Rock, AR and [3]NCCT, US EPA, Research Triangle Park, NC.*

Read-across remains a popular data gap filling technique within category and analogue approaches for regulatory purposes. Acceptance of read-across is an ongoing challenge with several efforts underway for identifying and addressing uncertainties. Here we demonstrate an algorithmic approach to facilitate read-across using ToxCast *in vitro* bioactivity data in conjunction with chemical descriptor information to predict *in vivo* outcomes in guideline testing studies from ToxRefDB. Over 3400 different chemical structure descriptors were generated for a set of 976 chemicals and supplemented with the outcomes from 821 *in vitro* assays. The read-across prediction for a given chemical was based on the similarity weighted endpoint outcomes of its nearest neighbors calculated using *in vitro* bioactivity and chemical structure descriptors, called Generalized Read Across (GenRA). GenRA is based on a computational approach for: (i) defining local validity domains using chemical and bioactivity descriptors, (ii) systematically deriving endpoint read-across predictions within these domains using similarity weighted activity of nearest neighbours, (iii) objectively evaluating predicted performance using tested chemicals, and (iv) assigning read-across predictions to untested chemicals along with estimates of uncertainty. We found *in vitro* bioactivity descriptors were often found to be more predictive of *in vivo* toxicity outcomes than chemical structure descriptors. We believe GenRA is an important first step in systematizing read-across prediction of toxicity and serves as a useful tool as part of a screening level hazard assessment for new untested chemicals. This abstract does not reflect US EPA policy.

PS 2162 Preliminary Evaluation of the Draft NIOSH Occupational Exposure Banding Protocol

C. Whittaker, S. Gilbert, L. McKernan and M. Seaton. *NIOSH, Cincinnati, OH.* Sponsor: S. Perumal Kuppusamy.

NIOSH recognizes that chemicals are being introduced at a rate that significantly outpaces occupational exposure limit (OEL) development. While NIOSH develops new OELs and updates existing OELs, guidance is needed for the thousands of chemicals that lack exposure limits. To that end, NIOSH is developing an occupational exposure banding (OEB) protocol to address the myriad unregulated chemicals in commerce. The protocol would sort chemicals into five air concentration bands based on toxicity. Chemicals with the lowest toxicity would be grouped in band A, while band E would include the most toxic chemicals. The proposed protocol uses a three-tiered evaluation system and gathers available toxicological data from preselected sources to assign the appropriate band or range of chemical concentrations. Important questions include the reliability of the protocol over a variety of chemical types and families and the reproducibility of the system across users. In a pilot testing of the draft OEB protocol, the concentration range corresponding to each band was compared with published OELs for 47 chemicals banded in Tier 1 and Tier 2 of the protocol. For 46 of the chemicals, the resulting bands were as or more protective than the published OEL. Only one chemical received a band less protective that the OEL. Preliminary comparisons of novel user experiences with the banding protocol indicated some inconsistencies in application of the protocol across users. Further analysis indicated that one of the primary reasons for the inconsistencies was the user not following the instructions. NIOSH staff are working to simplify and clarify the instructions to ameliorate that issue. [The findings and conclusions in this presentation are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.]

PS 2163 Development of a Master Database of Non-Cancer Threshold of Toxicological Concern and Potency Categorization Based on ToxPrint Chemotypes

C. Yang[1,5], K. Arvidson[9], M. Cheeseman[7], M. Cronin[4], S. Enoch[4], S. Escher[2], E. Fioravanzo[6], K. Jacobs[9], T. Steger-Hartmann[8], I. Tluczkiewicz[2], A. Tarkhov[5], J. Rathman[1], V. Vitcheva[1], A. Mostrag[1] and A. Worth[3]. *[1]Altamira LLC, Columbus, OH; [2]Fraunhofer (ITEM), Hanover, Germany; [3]Joint Research Centre, European Commission, Ispra, Italy; [4]Liverpool John Moores University, Liverpool, United Kingdom; [5]Molecular Networks GmbH, Erlangen, Germany; [6]S-IN, Vicenza, Italy; [7]Steptoe&Johnson, Washington, DC; [8]Investigational Toxicology, Bayer HealthCare, Berlin, Germany and [9]OFAS, US FDA CFSAN, College Park, MD.*

Threshold of toxicological concern (TTC) is one of the alternative approaches for the safety/risk assessment of food additives, metabolites of agrochemicals, and pharmaceutical impurities. In the COSMOS project, a new non-cancer TTC database was developed following a set of rigorous inclusion criteria and study reviews of the potent chemicals to expand the chemical space for cosmetics-related chemicals. The COSMOS TTC database integrated data from US FDA, EPA (ToxRefDB and IRIS), EU SCCS, EFSA, and Munro TTC, which was further merged with RepDose and HESS databases to create an initial master database of 2400 unique compounds with NO(A)EL/LO(A)EL values. The database was then classified by Cramer Classes, and each group was profiled using ToxPrint chemotypes. The potency of the ToxPrint chemotypes was compared by calculating z-scores as well as pairwise t-tests. For agro- and industrial chemicals, the potent classes (low NOAEL/LOAEL) include organohalides, nitrile, organosulfur, (thio)carbamates, carbocycles, conazole and triazole. For chemicals used in cosmetics, food, and consumer products, aromatic amines, secondary alkyl amines, nitro, ethanolamines were found to be potent. For all chemical use types, alcohols, carbohydrates, surfactants, and carboxylic acid are much less-potent. The chemical

space was also compared with the CPDB-based cancer TTC database. The ultimate goal is to develop structural categories that will discriminate potency through the chemotype analysis combined with mechanistic knowledge. The initial master database will be further augmented with pharmaceutical compounds and curated by enforcing the same study inclusion criteria used in the COSMOS TTC project to ultimately construct the master TTC database, the most extensive of its kind to date. This abstract does not reflect the policy of US FDA, JRC, or EC.

2164 Application of Gene Set Enrichment Analysis for Identification of Critical Gene Expression Networks for Application in Human Health Risk Assessment

J. Dean[2], B. Hawkins[2], M. Black[1], R. Thomas[3] and S. Wesselkamper[2]. *[1]The Hamner Institutes for Health Sciences, Research Triangle Park, NC; [2]US EPA/NCEA, Cincinnati, OH and [3]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC.*

The rate of new chemical development combined with a paucity of toxicity values for legacy chemicals presents a unique challenge for human health risk assessment. There is a clear need to develop new technologies and incorporate novel data streams into human health risk assessments to more efficiently inform derivation of toxicity values. One potential avenue of exploitation lies in the field of toxicogenomics and the application of gene expression analysis to chemical exposures. In this context, Gene Set Enrichment Analysis (GSEA) was employed to identify biologically-relevant transcriptional benchmark dose (BMD) values for 1,2,4-Tribromobenzene (TRBZ). Initially, Affymetrix gene expression data were extracted from rat liver samples collected at multiple time points from previously published dose-response exposure studies (GEO Accession: GSE45892). Unfiltered datasets from individual animals were scored for enrichment of biologically-derived gene expression signatures via a ranked running-sum statistic method. Preliminary results examining the enrichment of "Hallmark" gene sets (Broad Institute) revealed significant ($p<0.05$, $FDR<0.05$) and differential enrichment of molecular signaling pathways involved in the response to hepatocellular damage and subsequent liver-specific regenerative phenotypes, providing a novel basis to inform molecular characterization of disease-specific mechanism of action. Enriched "Hallmark" pathways were then used to determine relevant genes for BMD analysis and derivation of points of departure. In summary, the present study exemplifies how GSEA can be used to identify biologically relevant gene signaling networks among *in vivo* model systems of chemical exposure. The views expressed in this abstract are those of the authors and do not necessarily reflect the views and policies of the US EPA.

2165 A Next Generation Benchmark Dose Computation System

K. Shao[2] and A. Shapiro[1]. *[1]Independent Consultant, Durham, NC and [2]Department of Environmental Health, Indiana University, Bloomington, IN.*

Since the introduction of the benchmark dose (BMD) methodology, the Benchmark Dose Software (BMDS) published by the US Environmental Protection Agency (EPA) has played a key role in facilitating and promoting the BMD approach. BMDS uses maximum likelihood estimation methods to fit dose-response models and produce point estimates for model parameters, benchmark dose values, and other quantities of interest. As the mainstream regulatory risk assessment community is moving towards a probabilistic assessment framework, more robust methods are needed to provide quantitative estimates (such as distributional estimation) to support risk assessment and enable benefit-risk analysis. To address these needs and further the state of the science, a new system fully based on Bayesian statistics has been developed. This software application utilizes the Stan probabilistic programming language for model fitting using MCMC sampling for Bayesian inference. Modeling outputs include distributions of model-fit parameters, distributions of BMD estimates instead of point estimates of BMD/BMDL, and risk/response calculations for user-specified dose levels. Posterior model weights are used to not only evaluate model fitting performance but also to calculate model-weight averaged BMDs, instead of the conventional single-model selection paradigm using the Akaike information criterion (AIC). Further, the system also has the potential to incorporate additional knowledge as Bayesian priors to inform model fitting and BMD estimation. Here we present the new software system and the web-based interface which has been developed to enable users to conduct their own analyses.

2166 Challenges in Developing a Systematic Review Protocol for Environmental Contaminants

T. Greene[3], A. Franzen[3], L. Yost[2], N. Beck[1], R. Fensterheim[4] and R. Gentry[3]. *[1]American Chemistry Council, Washington, DC; [2]Environ Ramboll, Chicago, IL; [3]Environ Ramboll, Monroe, LA and [4]RegNet Environmental Services, Washington, DC.*

Government programs such as US EPA IRIS and NIEHS/NTP Report on Carcinogens are attempting to incorporate principals of systematic review into their standard processes for hazard identification and dose-response assessment. A key aspect of systematic review requires the development of a protocol prior to initiation of an assessment. The protocol is intended to assist with problem formulation and scoping and should lay out the specific approach that will be used for the assessment. In particular, a protocol should include: the specific questions that the systematic review will be designed to answer, the literature search strategy and plans for data collection, methods for evaluating data quality, (including study quality, risk of bias, and human relevance) and finally the approach for data analysis/evidence integration. An example protocol for the hazard identification and dose-response was developed. As part of the development process, methods that could significantly affect the subsequent decisions and choices for risk assessors were identified. While there is some general agreement in the environmental community regarding a robust literature search, other aspects of the protocol have not been well defined. For example, there is no accepted approach to assessing individual study quality and as such, alternative systems for quality evaluation and risk of bias were explored. The protocol presented improves upon the NIEHS/NTP approach but refines it by describing an approach that quantitatively evaluates each study (from all streams of evidence) for important study quality and risk of bias elements. This approach assists in the integration of information across streams of evidence, and also relies upon the studies that are of the highest quality, which should be the focus of risk assessment decisions. Funded by ACC ARASP.

2167 Validating the Use of DoCTER, a Natural Language Processing Tool, for Prioritizing Scientific Literature

M. Cawley[1], J. Wignall[2] and A. Varghese[1]. *[1]Env. Health Sci., ICF International, Durham, NC and [2]Env. Health Sci., ICF International, Fairfax, VA.*

Risk assessments on data rich chemicals or broad topics include the need to review large amounts of literature for purposes such as hazard identification, which necessitate the use of sophisticated methods to identify potentially relevant studies efficiently. DoCTER, or the Document Classification and Topic Extraction Resource, is a tool developed by ICF to prioritize scientific literature from large literature result sets such as these. DoCTER uses natural language processing (NLP) to classify text into any number of groups using statistical algorithms. For this effort we used unsupervised machine learning (no training dataset required) to cluster literature based on study titles and abstracts into groups using 6 approaches (2 statistical algorithms x 10, 20, or 30 groups). We also made use of a set of "known to be relevant" literature or "seeds" developed based on our past work identifying hazard ID literature. We removed all chemical-specific terms from the seed set rendering it chemical agnostic. We expected that literature associated with seed studies in all 6 DoCTER approaches would include a higher percentage of relevant results compared to literature that was common to fewer approaches. We tested this method on results from a non-chemical specific literature search. As expected, the proportion of relevant studies, including supporting studies, was highest in literature found using all 6 approaches (55% relevant) as compared to literature found using fewer approaches (45% relevant in 5 approaches). When we removed supporting studies from the analysis and only considered relevant hazard ID literature, the results were more striking (67% relevant in 6 approaches and 33% relevant for references found in 5 approaches). This significant difference in the proportion of relevant studies suggests that our unique approach of using NLP holds great promise for prioritizing scientific literature in an efficient and targeted way and offers an alternative to using a keyword-only approach to finding relevant literature.

2168 Text Analytics to Gain Insights on Chemical-Specific MOAs

P. Ross[2], A. Turley[1], A. Bordner[1], R. Blain[2] and W. Mendez[2]. [1]*Env. Health Sci., ICF International, Durham, NC and* [2]*Env. Health Sci., ICF International, Fairfax, VA.*

In the field of human health risk assessment, better understanding of the mechanism by which a chemical induces disease is critical to more informed dose-response modeling and evaluation of cumulative effects. Adverse Outcome Pathways (AOPs) can be used to more fully understand a chemical's mode of action (MOA) for a specific endpoint by identifying chemical-specific supporting evidence for each key event along the AOP. Data mining tools exist to enable faster review of evidence of chemical-induced gene activation and protein changes, but identifying supporting evidence for all key events, especially those occurring at the tissue level and above, still often requires time- and resource-intensive manual review of many (potentially thousands) of reports. Using arsenic as a case study, we explored the use of publicly available resources coupled with text mining techniques and innovative data management tools to streamline this process. We first identified approximately 5,000 articles describing mechanisms by which arsenic might cause disease and then used the disease pathway diagrams from the Kyoto Encyclopedia of Genes and Genome (KEGG) database as a starting point for organizing the data into hypothesized MOAs. Specifically, we extracted key events and their synonyms from the KEGG pathway for bladder cancer, and then fed these terms into text mining code to identify specific titles/abstracts that included each key event term. Prioritizing studies for review in this way reduced the number of studies needing hand curation and reduced the time spent reviewing non-relevant references, as expert review of the references confirmed the accuracy of the text mining results. The text mining also revealed the frequency and interactions of certain terms that appeared in the arsenic literature, indicating the degree to which certain key events might be important to describe potential modes of action for bladder cancer or where data gaps along the bladder cancer MOA might be. We used a data management tool, DRAGON, to organize the data throughout the process.

2169 Automated Toxicological Synonym Generation Using Neural Network-Based Language Models

A. Varghese[1], T. Hong[1] and P. Ross[2]. [1]*Env. Health Sci., ICF International, Durham, NC and* [2]*Env. Health Sci., ICF International, Fairfax, VA.*

Natural language processing-based automated document reading technologies are gaining increasing traction in the text-rich field of toxicology. Although supervised and unsupervised machine learning algorithms are efficient at classifying toxicological literature into broad categories, advanced keyword-based search algorithms are the most effective means of identifying literature related to nuanced topics such as finding evidence in support of computationally hypothesized adverse outcome pathways or modes of action. Popular web-search engines typically expand a user's search keywords to include automatically generated synonym lists to effect a more comprehensive web-search. In this study, the efficacy of similar synonym generation technology was tested in the context of toxicological literature search. A corpus of approximately 1 million abstracts was created from the toxicological literature to test the performance and precision of toxicological keyword synonym generation algorithms based on unsupervised neural network-based language models that rely on the distributional representation of words as mathematical vectors. The performance of two advanced word representation models were evaluated in the context of synonym and related terms generation for a set of genes and proteins frequently associated with toxicological impacts. Both algorithms are based on adaptations and simplifications of deep learning technologies from the field of natural language processing and do not require training based on previously classified data or synonym lists. The results of these automated analyses were compared against an expert judgment-derived set of synonyms and related terms, and, promisingly, showed greater than 75% agreement on average. The study examines the implications of corpus size on precision and proposes optimal standards of practice for the application of these algorithms. The potential use of these algorithms in other applications are discussed, including temporal trend analysis in the co-occurrence of toxicological keywords of interest, such as important genes and proteins.

2170 Acute Toxicity When Concentration Varies with Time: A Case Study with Carbon Monoxide Inhalation by Rats

L. M. Sweeney[3,4], M. R. Goodwin[1,4], R. A. James[1,4] and D. R. Sommerville[2]. [1]*CAMRIS, Bethesda, MD;* [2]*Edgewood Chemical Biological Command, Aberdeen Proving Ground, MD;* [3]*Henry M. Jackson Foundation, Bethesda, MD and* [4]*Naval Medical Research Unit Dayton, Wright Patterson AFB, OH.*

Exposure to time-varying concentrations of toxic compounds is the norm in both occupational settings and daily human life, in contrast to the almost exclusive use of constant concentration exposures in toxicity assessment. Surprisingly little has been done to systematically investigate the impact of variations in concentration vs. time on toxic outcomes. Acute lethality studies were conducted where male Sprague-Dawley rats inhaled carbon monoxide. Median lethality (LC50) of 10-, 20-, 40-, and 60-min continuous exposures was well described by the toxic load model (ten Berge's law, $k = (C^n) \times t$, where k is constant, C is test concentration, n is the chemical-specific toxic load exponent, and t is the exposure duration) with $n = 1.74$ (standard error = 0.1). Dose response-relationships for 1-hr exposures that included a recovery period between 10- or 20-min pulses showed greater similarity to the dose-response relationships of continuous exposures with equivalent pulse duration (10 or 20 min), rather than a 60-min exposure with equivalent time-weighted average concentrations. Also, whereas the LC50s for 20- and 40-min continuous exposure were 6517 and 4370 ppm, respectively, the LC50 for 40 min exposure when divided into two 20-min, equal concentration pulses with a 20-min recovery period was 5689 ppm. This finding indicates that the two pulses both contributed to lethality (lower LC50 than one 20-min pulse), but that the non-exposure interval allowed rats to recover, compared to equal total exposure if administered with no interruption (higher LC50 than one 40-min pulse). These findings show that fluctuations or interruptions in exposure over a short time scale (60 minutes or less) can have a substantial impact on outcomes for chemicals with toxic load exponents not equal to 1, and thus indicate a need for high-resolution monitoring data to aid interpretation of resulting outcomes.

2171 Development of Advanced 3D-Models with Human Primary Bronchial Epithelial Cells (PBEC) for Exposure to Nanoparticles Present in Air Pollution

L. Palmberg[3], J. Ji[3], A. Hedlin[3], M. Börjel[2,3], V. Kessler[1], G. Seisenbaeva[1] and P. Gerde[2,3]. [1]*Department of Chemistry and Biotechnology, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2]*Inhalation Sciences Sweden AB, KFC, Huddinge, Sweden and* [3]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

Aim: Exposures to inhaled nanoparticles (NP), with the lung as the primary target, are of great concern. Our models are unique in combining the use of human PBEC with quality exposures, in a strategy that mimics realistic exposures of the human airway wall. For both healthy subjects and subjects with respiratory diseases, our chosen test substrates of highly dispersed NP present in polluted ambient air are of clinical importance. With further development and validation, these models could form part of an *in vitro* testing strategy to reduce the requirement for animal inhalation studies. Methods: Confocal-scanning- and transmission electron microscopy were used to document the appearance of PBEC grown under air-liquid interface culture condition. Both models were exposed to clean air or custom synthesized palladium (Pd) NP at three different concentrations applying the XposeALI module of the PreciseInhale system -200ng, 400ng and 600ng per insert- then incubated for 24 hrs. Both apical (AM) and basal medium (BM) were collected. The release of chemo-attractants (CXCL8), Matrix metallopeptidase 9 (MMP-9) and Club cell protein (CC16) was detected by ELISA. Cell viability and apoptosis were analyzed with trypan blue and the annexin V-PE/7-AAD kit, respectively. Results: By using PBEC and fibroblasts we established 3D-models mimicking both normal and chronic bronchitis-like mucosa. In addition, we succeeded in combining the 3D-models with controlled aerosol exposures using the XposeALI exposure module. Exposure to Pd NP induced an increased secretion of IL-8, where chronic bronchitis-like models released significantly more IL-8 than normal models. The levels of IL-8 in BM and AM were in the same range, but MMP-9 was much higher in the AM compared to BM. The viability of the models was > 90% and the apoptotic rate between 1% -30%. Conclusion: By using primary human bronchial epithelial cells we have developed unique airway wall models, expressing either normal or chronic bronchitis-like morphologies. These sophisticated models with primary human cells mimic the *in vivo* situation and provide useful and relevant tools when studying the effects of NP present in air pollution.

2172 Organotypic Three-Dimensional Culture Model Engineered to Recapitulate Morphogenetic Fusion Using Human Cells

D. G. Belair, C. J. Wolf, K. Das, A. Watkins and B. D. Abbott. *ORD/NHEERL/TAD, US EPA, Research Triangle Park, NC.*

Tissue fusion during early mammalian development requires coordination of multiple cell types, the extracellular matrix, and complex signaling pathways. Fusion events during processes including heart development, neural tube closure, and palatal fusion are dependent on signaling pathways elucidated using gene knockout mouse models. A broad analysis of literature, ToxRefDB, and ToxCast identified 63 chemicals that are related to cleft palate. However, the influence of these putative teratogens on human palatal fusion has not been studied due to the lack of *in vitro* models. We sought to engineer the stratified mesenchymal and epithelial structure of the developing palate *in vitro* via organotypic culture of human mesenchymal stem cell (hMSC) spheroids coated with a single layer of human primary epidermal keratinocytes (hPEKp). hMSC spheroids exhibited uniform size over time (175 ± 21 µm mean diameter) proportional to starting cell density. Further, we developed a novel procedure to coat hMSC spheroids homogeneously with a single layer of hPEKp cells using a seeding ratio of 0.1-0.2 hPEKp per hMSC, and hMSC/hPEKp spheroids expressed mesenchymal markers (vim+, CD44+, CD105+, CD34-) and epithelial markers (krt17+, itga6+) via qRT-PCR. Analysis of adverse outcome pathways related to palate fusion points to an EGF/TGFβ3 switch that could be a target for cleft palate teratogens, and both egf and egfr were expressed by hMSC/hPEKp spheres. Finally, hMSCs and hPEKp cells expressed a model protein, GFP, using non-viral transfection. We hypothesize that hMSC/hPEKp spheroids together with genetic engineering approaches will enable interrogation of signaling pathways crucial to morphogenetic fusion for predictive toxicology. The organotypic culture model described here can be broadly applied to model morphogenetic fusion of other stratified mesenchymal and epithelial tissues. This abstract does not necessarily reflect US EPA policy.

2173 Characterization of 3D HepaRG Microtissues for Improved Adme/tox Predictions

K. Roessger[2], V. Shevchenko[1], C. Chesne[1], S. Messner[2] and J. M. Kelm[2]. [1]*Biopredic International Ltd., Saint-Grégoire, France and* [2]*InSphero AG, Schlieren, Switzerland.*

The human hepatic cell line HepaRG is a well-known alternative to primary hepatocytes. Similar to primary human hepatocytes, HepaRG exhibit phase I+II metabolism, but retain the advantages of cell lines such as high reproducibility and infinite availability. In this study the properties of HepaRG, cultured as 3D microtissues, were characterized. 3D HepaRG microtissues were produced in ultra-low attachment plates at a size of 250 µm. The HepaRG microtissues were viable and stable for least 3 weeks of culture. The liver functionality was proven by detection of albumin secretion (0.8 pg/cell*h) and basal CYP-activity (CYP1A2, CYP2B6, CYP2C19, CYP2C9, CYP2D6 and CYP3A4). In addition, responsiveness toward CYP-inducers Omeprazol (8.1-fold of CYP1A2), Rifampicin (4.7-fold of CYP3A4) and Phenobarbital (1.9-fold of CYP2B6) was demonstrated. Histological sections indicated presence of a healthy tissue with high glycogen incorporation as well as polarized expression of the bile acid transporter MRP-2. Furthermore, we have developed a serum-free toxicity testing medium for long-term toxicity studies. 3D HepaRG microtissues were sensitive to drug-induced liver injury (DILI) causing compounds Tolcapone, Chlorpromazine and Ketoconazole, whereas non-DILI compounds Entacapone and Ambrisentan were not toxic to HepaRG-microtissues. As drug-induced mitochondrial dysfunction plays a major role in DILI, we have also examined the applicability of the developed 3D HepaRG microtissue model for testing mitochondrial toxicity, by using the Seahorse Mito Stress Test. With this test, the mitochondrial toxicants Amiodarone and valproic acid were correctly detected, whereas Amantadine and Nilotinib were correctly negative for mitochondrial toxicity. In summary, the obtained results indicate that 3D HepaRG microtissues represent a robust organotypic liver microtissue model and are therefore suitable for various applications in drug-induced toxicity detection.

2174 Assessment of Androgenic and Anti-Androgenic Activity Using RWPE-1 Prostate Microtissues

M. Vantangoli[1], S. Madnick[1], S. Wilson[1], M. Dent[2], P. Carmichael[2] and K. Boekelheide[1]. [1]*Pathology and Laboratory Medicine, Brown University, Providence, RI and* [2]*Safety & Environmental Assurance Centre, Unilever, Sharnbrook, United Kingdom.*

Current methods to evaluate the effects of compounds on the endocrine and reproductive system are limited by low-throughput and costly *in vivo* experiments that lack relevance to human exposure and disease. A large area of concern is the potential for compounds to interfere with androgen signaling, which may have effects on reproductive tract development and fertility. We demonstrate that in a scaffold-free agarose hydrogel system, RWPE-1 prostate epithelial cells derived from normal adult prostate form differentiated microtissues when grown in complete growth media consisting of keratinocyte serum free media (KSFM) supplemented with epidermal growth factor (EGF) and bovine pituitary extract (BPE). RWPE-1 microtissue morphology is sensitive to alterations in the growth media, as well as testosterone treatment. When grown in KFSM supplemented with EGF, squamous differentiation of RWPE-1 microtissues was induced within 7 days. In KSFM supplemented with EGF and testosterone, RWPE-1 microtissues demonstrated increased proliferation and increased cellular debris. Using this model, morphological and molecular endpoints can be assessed to determine the potential androgenic and anti-androgenic activities of environmental chemicals within this system, providing more complete data sets to evaluate the potential adaptive and adverse responses of RWPE-1 microtissues.

2175 Effect of Acclimation Conditions on 3D Human *In Vitro* ALI Airway Models: Exposure-Induced Inflammatory Response and Sampling Location-Specific Results

H. P. Behrsing[1], L. E. Haswell[2], A. M. Hilberer[1], D. W. Sheehan[1], E. A. Sly[1], M. Gaça[2] and H. A. Raabe[1]. [1]*Institute for In Vitro Sciences, Inc., Gaithersburg, MD and* [2]*R&D Centre, British American Tobacco, Southampton, SO15 8TL, United Kingdom.*

Reconstructed human airway (RHuA) tissues are 3D organotypic models that feature relevant cell types and more accurately reflect the pulmonary airway *in vivo*. We conducted a pilot study to characterize and test acclimation conditions on RHuA tissue inflammatory responses. MatTek EpiAirway tissues were received and acclimated for 24 or 48hr in medium ± 2 µM hydrocortisone (HC). Triplicate tissues were treated apically with 15 µg/mL Poly I:C or 5 µg/mL lipopolysaccharide for 24hr as reference agents. Apical rinse, tissue lysate, and basal medium samples were collected. Total protein, LDH, IL-6, IL-8, and IP-10 were assayed in single-plex, followed by a 30-plex human cytokine evaluation. Single-plex results from the 48hr HC(+) acclimation indicated a 6-fold greater average biomarker increase compared with 24hr HC(+) or HC(-) groups following challenge . Markers were up to ~5-fold higher in apical rinse and tissue lysates compared to basal medium. IP-10 exhibited the greatest increase over control (160-fold in tissue lysate; 139-fold in apical rinse, and 25-fold in basal medium) following Poly I:C challenge. The 30-plex assay verified and expanded the quantitation of inflammatory responses. Both reference agents elicited distinct proinflammatory, cytokine, and chemokine marker release profiles that defined a material-specific "signature" of inflammation. Optimized tissue acclimation and appropriate sampling from various RHuA compartments following apical exposures should be considered during study design. RHuA are increasingly used to assess inhalation exposures, such as cigarette smoke and E-cigarette vapors. Characterizing multiple sample compartments following apical challenge will identify exposure specific inflammatory responses. These responses may offer increased sensitivity for comparisons of cigarettes and novel products such as e-cigarettes.

2176 Human Relevant Intra-Laboratory Validated *In Vitro* Vasculo/Angiogenesis Test

T. Heinonen, O. Huttala and T. Toimela. *FICAM, University of Tampere, Tampere, Finland.* Sponsor: T. Knudsen.

Formation of vascular network is a crucial process during embryonic development, and it also contributes to the pathogenesis of numerous disorders such as cancer and diabetes mellitus. One of the principal teratogenic mechanisms suspected to be associated with medication is vascular disruption (van Gelder et al.,Therapie 69(1), 13-24. 2014). Vascular network is formed either by vasculogenesis (*in situ* differentiation and growth of blood vessels) and angiogenesis (growth of new blood vessels from the preexisting ones). The vasculogenesis/angiogenesis test developed in FICAM utilizes primary human adipose stem cells and primary

human umbilical cord vein endothelial cells. The 3D coculture formed contains 3D endothelial tubule structure (vWF) with lumen, younger and more mature vessels, basement membrane (colIV), adherens junctions (ve-cadherin, occludin) between endothelia, pericytes (PDGFRb, SMMHc, SMA) and endothelial cells characterized by immunostaining and electron microscopy. Further, the maturation process covering vasculogenesis and angiogenesis was confirmed by measuring the expression of the key marker genes (e.g. ANGPT1/2, FGF2, VEGF, PDGF, VEGFR1/2, TIE-2, PDGFRb). The test passed successfully intra-laboratory validation according to the OECD 34 guideline: reproducibility, repeatability and relevance were proven. There was no statistical difference between the results of different technicians (P=0.44, ANOVA) or different repeats of the same technician (CV<15%). The relevance to inhibit tubule formation was shown with drugs with published human data e.g. Erlotinib, Levamisole, 2-methoxyestradiol, acetylsalicylic acid, anti-VEGF, Cladribine, and Doxorubicin resulting in a good concordance (Pearson R=0.6921). This indicates that this human cell based test has potential be used as predictive *in vitro* test in assessing vascular disrupting potential of chemical substances in man. The applicability covers development of vascular-disrupting drugs as well as testing of chemicals for their potential to disrupt vasculature formation during embryogenesis.

PS 2177 N-(2-Aminoethyl) Ethanolamine Induced Morphological, Biochemical and Biophysical Alterations in Vascular Matrix Associated with Dissecting Aortic Aneurysm

Z. Chen[2], Y. Xu[2], P. Bujalowski[2], A. F. Oberhauser[2] and P. J. Boor[2]. [1]*Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX and* [2]*Department of Neuroscience and Cell Biology, Sealy Center for Structural Biology and Molecular Bioph, The University of Texas Medical Branch, Galveston, TX;* [3]*Pathology, The University of Texas Medical Branch, Galveston, TX.* Sponsor: F. Khan.

Dissecting aortic aneurysm (DAA) is an extended tear in the wall of the aorta along the plane of the vascular media. We recently established a chemical-induced animal model of DAA. While the animal model remains a crucial testing ground for studying the mechanisms that underlie DAA, *in vitro* approaches may offer many advantages. Literature using *in vitro* ECM to study DAA and other vascular phenomenon is scanty. In this study, neonatal aortic vascular smooth muscle cells (VSMC) and timed pregnant Sprague-Dawley rat dams were treated with N-(2-Aminoethyl) Ethanolamine (AEEA), which causes DAA *in vivo* in offspring. Extracellular matrix (ECM) changes produced by VSMC *in vitro* were morphologically detailed with scanning electron microscopy (SEM); and biochemical changes in cells and ECM produced by VSMCs were defined by Western blotting. Biophysical aberrations in collagen extracted from both fetal rat aortas and the ECM produced by VSMC were studied with atomic force microscopy (AFM). Structural disruption and irregularities were observed in ECM made by VSMCs treated with AEEA by SEM. Western blotting showed that collagen type I made by AEEA treated VSMC was much more extractable, accompanied by a decrease in pellet size after urea buffer extraction. AFM showed that collagen samples extracted from the fetal rat aortas of the AEEA treated dam, and from the ECM made *in vitro* by AEEA exposed VSMC, were stiffer, or more brittle, indicating that the three-dimensional organization associated with vessel strength and elasticity was altered by AEEA exposure. Our results show that AEEA causes significant morphological, biological and biomechanical alterations in the ECM. These *in vitro* and *in vivo* strategies are advantageous in elucidating the underlying mechanisms of DAA. Key words: dissecting aortic aneurysm, vascular smooth muscle cells, ECM, AEEA, collagen.

PS 2178 High Density Human Stem Cell Micromass to Model Teratogen-Induced Limb Malformations

S. Yu, J. Tan, K. Clark, P. G. Alexander and R. Tuan. *Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA.* Sponsor: L. Vernetti.

Limb malformations are a major feature of exposure to many developmental teratogens. Embryonic limb mesenchyme high density micromass culture is an excellent system for studying skeletal tissue development *in vitro*. We have developed micromass cultures with human adult bone marrow-derived mesenchymal stem cells (hBMSC), a more homogenous cell population, that can undergo chondrogenesis and chondrocyte hypertrophy in a predictable and physiological manner. In this study, we challenge hBMSC-based micromass with three toxicants, thalidomide, warfarin and mifepristone, to test the possibility that this hBMSC-based culture may be used to study the effects of teratogenic compounds on limb development. hBMSCs micromass were induced to undergo chondrogenesis in TGF-β1 containing medium. On day 10, cultures were either analyzed for chondrogenic differentiation or induced to undergo hypertrophy by TGF- β1 withdrawal and T3 supplementation. Candidate teratogens were added throughout the culture period and the effect on chondrogenic and hypertophic markers were assayed histologically and by qRT-PCR as a function of dose. Control micromasses demonstrated robust chondrogenesis and hypertrophy, indicated by increased Alcian Blue staining and alkaline phosphatase (ALP), activity, respectively, and stage-specific gene expression. Thalidomide did not show any detectable impact on chondrogenesis and hypertrophy at any dose. Warfarin had minimal impact on chondrogenic gene expression but a significant effect on hypertrophic gene expression, matrix elaboration and ALP activity. Mifepristone significantly reduced chondrogenic and subsequent hypertrophic gene expression. The effects of both warfarin and mifepristone occurred at doses near reported maternal serum levels. The 3 toxicants tested affected hBMSC micromasses in ways related to their mechanism of action. Thalidomide, an inhibitor of angiogenesis, did not show any detectable impact on chondrogenesis or hypertrophy at any dose in this avascular model Warfarin, an inhibitor of post-translational γ-carboxylation critical for cartilage matrix production, affected mainly hypertrophy. Mifepristone, a glucocorticoid antagonist, strongly inhibited both chondrogenesis and hypertrophy. We conclude that high density, 3-dimensional hBMSC-based micromass cultures represent a reproducible and controlled model to screen for potential limb teratogens.

PS 2179 Metabolically Competent HepaRG Spheroid Model for *In Vitro* Toxicology Studies

S. Ramaiahgari, S. Waidyanatha, W. Boyd, M. DeVito, R. Paules and S. Ferguson. *NIH/NIEHS, DNTP, Durham, NC.*

Chemicals are de-toxified or converted to toxic metabolites by xenobiotic metabolism enzymes. *In vitro* liver models using proliferating cell lines or two-dimensional (2D) monolayer cultures generally lack sufficient xenobiotic metabolic competence thereby limiting their utility for toxicity evaluations. Though suspensions of primary human hepatocytes (PHHs) are considered the 'gold standard' for xenobiotic metabolism studies, their limited longevity, rapid de-differentiation *ex vivo*, and substantial donor variability are barriers in toxicology screening. HepaRG cells represent a promising alternative comprised of bi-potent progenitor cells that differentiate into co-cultures of hepatocyte- and cholangiocyte-like cells. To date most HepaRG research employ standard 2D culture formats requiring high concentrations of dimethyl sulfoxide (DMSO) to maintain physiologically-relevant levels of metabolic competence. Herein we have developed a three-dimensional (3D) culture model of HepaRG spheroids that eliminate the need for DMSO and maintain metabolic competence for up to 28 days in a 96/ 384- microplate format. These spheroids show several hallmarks of polarized hepatocytes with distinct apico-basal domains and their functions. Assessment of xenobiotic metabolism competence with clinical substrates of CYP1A2, CYP2B6 and CYP3A4 produced robust levels of liver enzymes that are within the ranges produced with PHH suspensions and a 2 to 20-fold higher than median activities observed with 2D cultures of PHHs or HepaRG. Spheroid size, time in culture and culture media were important factors affecting basal xenobiotic metabolism and inducibility with activators of hepatic nuclear receptors AhR, CAR and PXR. Repeated exposure studies showed differential sensitivity in identifying compounds that cause metabolism-dependent liver injury. This platform represents a promising new tool for the evaluation of liver toxicity potential incorporating 3D cell biology into toxicology screening.

PS 2180 Quantifiable 3D *In Vitro* Vasculogenesis Assay Emphasizes Importance of Cell-Matrix Interactions When Screening Putative Developmental Toxins

S. N. Thiede, N. Bajaj, K. Buno and S. Voytik-Harbin. *Biomedical Engineering, Purdue University, West Lafayette, IN.* Sponsor: J. Freeman.

3D *in vitro* human cell-based tissue systems incorporating both cell-cell and cell-extracellular matrix (ECM) interactions have promise to provide high-content, physiologically-relevant information on toxin mechanism and an accurate assessment of chemical toxicity before proceeding to *in vivo* testing. Vasculogenesis, the *de novo* formation of blood vessels from precursor cells, plays an essential role in embryonic development. As such, disruption of vasculogenesis can serve as an indicator for developmental toxicity. Recently, a 3D *in vitro* model of vasculogenesis, involving a type I collagen oligomer matrix and human endothelial colony forming cells, was described to induce formation and maintenance of lumenized blood vessel networks beyond 14 days. Here, we extend this work by validating the utility of this *in vitro* model for predictive,

high-content screening of developmental toxins using known vasculogenesis modulators (imazamox, thalidomide, 2-methoxyestradiol, levamisole). Insight into how each chemical affected the complex vasculogenesis process was elucidated by analyzing cytotoxicity, differences in 3D vessel morphogenesis, and vessel stabilization or regression at progressive time points. Initial cell health was compared between 2D and 3D assays at 24 hours with alamarBlue. Differences in 3D vessel morphogenesis at early (48 hour) and late (4 day) stages were qualitatively and quantitatively assessed through confocal microscopy and analysis using Imaris 3D imaging software. Based upon volumetric and morphometric analysis of 3D vessel network formation, vessel morphogenesis profiles for each chemical-treated tissue construct were categorized as 1) extensive vessel networks 2) small vessel networks 3) stunted vessels or 4) quiescent cells. Vessel stabilization or regression was measured at 4 days by evaluating co-localization of vessels and deposited basement membrane. Although cytotoxicity from both 2D and 3D assays indicated greater than 75% cell viability, distinct 3D vessel morphogenesis profiles and patterns in basement membrane deposition were observed for the chemicals tested. These results suggest that the 3D *in vitro* vasculogenesis assay, which recapitulates 3D cell-ECM interactions, may serve as a predictive high-content screening tool for developmental toxins, providing a low-cost alternative to *in vivo* models.

2181 C57BL/6 Fetal Mouse Midbrain Micromass Culture: A Case Study of Silver Nanoparticles

J. J. Park, B. Weldon, J. H. Park, W. Cruikshank, T. Workman, S. Hong, T. Ward, W. Griffith, T. J. Kavanagh and E. M. Faustman. *Department of Environmental & Occupational Health Sciences, University of Washington, Seattle, WA.*

Of 80,000 US Environmental Protection Agency (EPA) registered chemicals, only about 200 are tested for neurotoxicity (Miodovnik, 2011). In order to reduce the number of research animals while screening toxicity of potential neurotoxicants, *in vitro* rat embryonic cell micromass was modified and applied in this study. These silver nanoparticles (AgNPs) with two different sizes–20 and 110nm–were used as an example compounds due to their wide use in consumer products, medical devices, and in other forms of biotechnology. Their antimicrobial properties as well as their potential as neurotoxicants are under study. Gestational day 11 C57BL/6J mouse midbrain cells were isolated and plated in high density micromass format, and maintained for 22 days *in vitro* (DIV). To characterize the mouse micromass system, morphology and haematoxylin staining were utilized, demonstrating the extent of neuronal differentiation in our culture over time. Intensity of haematoxylin suggested linearly increasing presence of neuronal differentiation. Western blotting markers of proliferation (nestin, PCNA) and differentiation (β-tubulin, MAP-2, EN1) in the micromass culture were at its highest at DIV 4-6 and at DIV 6-8, respectively. Migration has also been observed *in vitro*. Immunohistochemistry showed concordant results with western blotting. Preliminary cytotoxicity data of AgNPs indicate a dose- response relationship and show significant increases in cell death in neuronal cells when exposed to 20nm AgCitrate at Day 15 and 22 *in vitro*. Similar results were observed in 110nm AgCitrate particles. No significant increases in cytotoxicity were observed on Day 8 *in vitro*. Together, this *in vitro* mouse midbrain micromass culture model will contribute significant information to current literature as the dynamics of neurodevelopment in the system is analogous to normal *in vivo* development and may be utilized to screen neurodevelopmental toxicity including AgNPs. Additionally, differences in response due to genetic variability may be studied with this model using various mouse strains. This work was done in collaboration with the NCNHIR Consortium and supported by NIH/NIEHS grants U19ES019545, P30ES07033 and RD835.

2182 Development of a 3D *In Vitro* Model for the Assessment of Repeated Dose Hepatotoxicity

A. Riu, S. Emery, R. Note, S. Teissier and J. Cotovio. *L'Oréal, Aulnay-sous-Bois, France.* Sponsor: E. Dufour.

Concerns about long-term repeated-dose toxicity assessment have been raised since the ban on animal testing for all cosmetic ingredients. Therefore, the development of innovative *in vitro* methods that are able to reproduce the physiological functionality of specific human organs has become essential and must continue in order to face the current challenges for risk assessment purposes. The screening of legacy data show that liver was the most targeted organ by cosmetic ingredients in repeated dose toxicity studies *in vivo* (Vinken et al. Arch. Toxicol 2012). Hence, we have developed a 3D spheroid assay based on HepG2 cells that we manage to maintain for more than 28 days to assess long-term hepatotoxicity of new cosmetic ingredients. The viability and the functionality of the system were monitored over a 28-day period. Because the liver is the metabolic engine of the body, we then characterized phase I and phase II drug metabolism enzymes, drug transporters and xenobiotic receptors expression as well as CYP450 activities and albumin secretion. An increase in liver-specific markers expression was observed in HepG2 spheroids compared to 2D culture. To validate the model, we used acetaminophen (APAP) which needs to undergo metabolic activation to induce its well-known hepatotoxic effect. Liver spheroids treated for 22 days with repeated exposures showed a sensitivity to APAP close to the non-observed adverse effect level found in rat *in vivo*, indicating a mechanistic toxic response over time. A larger set of compounds will be evaluated to better validate the model and expand its applicability domain. Our assay based on HepG2 cells cultivated as spheroids improved the metabolic activities required for a physiological functionality of the liver. This cost-effective model could be promising for a further screening of new cosmetic ingredients for long-term hepatotoxicity effect.

2183 Molecular Initiating Events in Toxicity Pathways Using Organotypic Human *In Vitro* Models

P. Fowler, S. Scott and P. Russell. *SEAC, Unilever, Bedford, United Kingdom.* Sponsor: P. Carmichael.

Within the new paradigms for toxicology risk assessment, understanding the mechanisms and interactions between chemical exposure and biology is paramount in being able to make pragmatic decisions on safety. This shifts focus away from traditional apical endpoint toxicology and leads to judgements based on sound mechanistic understanding. Pathways-based approaches such as AOP's are proposed as a framework for developing new approaches to assessing risk from chemical exposure. Key to these approaches is the molecular initiating event (MIE), which has been defined as the initial interaction between a molecule and a biomolecule or biosystem that can be linked to an outcome via a pathway 1. Of equal importance is the need to represent human biology accurately enough in *in vitro* models to allow meaningful mechanistic interactions. Such approaches capitalize on technologies that allow the user to interrogate multiple molecular pathways at the transcription and translational level. The present study aims to develop and refine appropriate human organotypic *in vitro* models, using a 3D Cardiac spheroid model comprising iPSC human cardiomyocytes, fibroblasts and endothelial cells as an initial case study, linking an understanding of MIEs to changes in molecular pathways to tissue level toxicity. A combination of novel tissue culture methods and molecular level analysis techniques have been employed. The 3D cardiac model has been exposed to Cyclophosphamide at physiologically relevant concentrations, the transcriptome and proteome of this system is mined for appropriate changes in biological pathways. This data is then mapped to the levels at which changes are seen biologically (metabolic efficiency, organelle health, membrane permeability) and which are indicative of downstream toxicity. Links between MIE's, molecular pathways and relevant toxicological effects to human organs, when combined with exposure predictions, will increase the mechanistic understanding for the risk assessment of chemicals. 1. Allen, T.E., Goodman, J.M., Gutsell, S., Russell, P.J. (2014) Defining molecular initiating events in the adverse outcome pathway framework for risk assessment. Chemical Research in Toxicology; 27 , 2100-2112

2184 3D Networks of iPSC-Derived Neurons and Glia for High-Throughput Neurotoxicity Screening

R. van Vught. *Mimetas, Leiden, Netherlands.* Sponsor: F. Russel.

The assessment of neurotoxicity remains a major scientific challenge due to the complexity of the central nervous system. Current strategies to evaluate toxicity of drugs and chemicals are predominantly based on *ex vivo* or *in vivo* animal studies. These models have limited predictability for neurotoxicity in humans and are not amenable to high-throughput testing. In order to overcome these limitations we are developing a neurotoxicity model based on iPSC-derived neurons in OrganoPlatesTM(1, 2). This microfluidic platform enables high-throughput screening of miniaturized organ models. A mixed population of human iPSC-derived neurons consisting of GABAergic and glutamatergic neurons with supporting astrocytes was cultured in 3D, closely representing the physiology of the human brain. As a part of the validation, proper network formation was observed by neuron-specific immunostainings and neuronal electrophysiology was analyzed by a calcium sensitive dye indicating spontaneous neuronal firing. Additionally, we investigated the dose-response neurotoxic effects of methylmercury and endosulfan on neuronal viability. The OrganoPlate™ platform enables real time analysis of neurotoxic effects of compounds in high-throughput. This iPSC-derived neuronal model can be used to refine animal experiments and has the potential to better predict adverse effect in humans and hence to improve clinical development success. [1] Trietsch, S.J., Israels, G.D., Joore, J., Hankemeier, T., Vulto, P. , Microfluidic titer plate for strat-

ified 3D cell culture, Lab Chip, 2013 vol. 13, no. 18, pp. 3548-54 [2] E. Lucumi Moreno, S. Hachi, K. Hemmer, S. J. Trietsch, A. S. Baumuratov, T. Hankemeier, P. Vulto, J. C. Schwamborn, R. M. T. Fleming, Differentiation of neuroepithelial stem cells into functional dopaminergic neurons in 3D microfluidic cell culture, Lab Chip, 2015, DOI: 10.1039/C5LC00180C

PS 2185 Developmental Neurotoxicity of Flame Retardants Using a Rat Primary Three-Dimensional Organotypic *In Vitro* Model

R. C. Sa[1,3], O. C. Ulker[2], L. Smirnova[1], T. Hartung[1] and H. Hogberg[1]. [1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD;* [2]*University of Ankara, Ankara, Turkey and* [3]*Department of Physiology and Pathology, Federal University of Paraiba, Joao Pessoa, Brazil.*

There is concern that flame retardants (FR) can exert potential adverse effects on children's health. In this study the developmental neurotoxicity (DNT) of one classical FR (tetrabromodiphenyl ether - BDE-47) and four replacements (isopropylated phenyl phosphate - IPP, triphenyl phosphate - TPP, 2,2'4,4'- tetrabromodiphenyl ether - BDE-47, isodecyl diphenyl phosphate - IDDP, and tricresyl phosphate - TCP) were investigated using a rat primary three-dimensional organotypic *in vitro* model. The 3D organoids were exposed at 7 days *in vitro* (DIV) up to 21 DIV to cover the most critical neurodevelopmental processes. Cell viability was determined using the resazurin assay and showed concentration-dependent effects with the percentage of live cells decreasing at higher concentrations and increased time (DIV 14 vs. DIV 21). Sub-cytotoxic concentrations (0.1, 1, and 5 µM) were selected for quantitative Real-Time RT-PCR analysis. Treatment with FR significantly modified expression of the selected genes: NF-200, Synapsin I, GABAA-R and NMDA-R (neuronal markers) were decreased, while GFAP, S100B, (astrocytic markers) and Nestin (precursor marker) were increased, indicating neural damage and activation of glial cells. Furthermore, the expression of IL-6 and TNF-α was also increased, suggesting an inflammatory response. The concentration-response effects at the mRNA levels, in this 3D model, show that gene expression can be a useful tool to alert of possible DNT. Glial cells, microglia and astrocytes are immune effectors, that exert a neuro-protective role in the brain, but their activation can result in enhanced production of cytokines (IL-6, TNF-α), which contribute to severe neuro-inflammation and neurodegeneration. Funded by US FDA #U01FD004230, 1CNPq Post-doctoral grant and NTP, NIH

PS 2186 *In Vitro* Assessment of Carcinogenic Risk for PAHs Using a 3D Lung Epithelial Model

L. K. Siddens, B. M. Tippman, K. L. Halvorson, C. V. Loehr and S. C. Tilton. *College of Veterinary Medicine, Oregon State University, Corvallis, OR; Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

One of the most difficult challenges for risk assessment is prediction and evaluation of health hazards from environmental chemical mixtures. Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental pollutants due to their prominence in mixtures of concern, including known/probable human carcinogens such as air pollution, diesel exhaust, cigarette smoke and coal tar. A growing need exists to establish better *in vitro* and computational models for screening chemicals and environmentally relevant mixtures for purposes of hazard assessment and prioritization for additional testing. Previously, we developed a modeling approach to predict the carcinogenic potential of PAH mixtures based on bioactivity profiles derived from global transcriptional analysis from mice *in vivo*. To improve both the application of our approach for high-throughput screening and the predictive capability for humans, we are systematically evaluating cytotoxicity, genotoxicity and gene expression associated with carcinogenic risk of PAHs in a highly differentiated, metabolically active *in vitro* human 3D bronchial epithelial cell (HBEC) model. In particular, benzo[a]pyrene (BAP) and dibenzo[def,p]chrysene (DBC), which we have previously identified to function through different mechanisms of action in skin, show differential gene expression in HBECs consistent with our *in vivo* data. We observed significant induction of arylhydrocarbon receptor-mediated expression of the metabolizing enzymes CYP1A1, CYP1B1 and ALDH3A1 in HBECs by qRT-PCR with 100 ug/ml BAP, but not with DBC at concentrations up to 5 ug/ml after 24 hrs. Treatment of HBECs with BAP (5 - 500 ug/ml) and DBC (0.05 - 5 ug/ml) for 24 hours resulted <15% cytotoxicity at any concentration as measured by MTT and MTS tetrazolium reduction assays. These data are consistent with our prior observations *in vivo* and indicate that BAP and DBC also function through unique mechanisms in HBECs. Our current data support the continued evaluation of HBECs for mechanistic-based predictions of carcinogenic risk.

PS 2187 Microfluidic 4-Organ-Chip Connecting Human Liver, Intestine, Kidney and Skin Equivalents to Approach ADME Profiling

I. Maschmeyer[1], A. K. Lorenz[1], J. Hübner[1], A. P. Ramme[1], R. Lauster[2] and U. Marx[1]. [1]*TissUse GmbH, Berlin, Germany and* [2]*TU Berlin, Berlin, Germany.* Sponsor: P. Hayden.

Ensuring good absorption, distribution, metabolism, excretion and toxicity (ADMET) properties is crucial to analyse if a drug reached its intended target and had a therapeutic effect without causing unacceptable toxicities. However, current *in vitro* models are lacking a systemic approach and therefore fail to predict the interaction of metabolites between organ cultures. Here, we present a new multi-organ approach to overcome this problem. Four human organ equivalents were combined by a microfluidic flow in a bioreactor the size of a microscopic slide. The 4-Organ-Chip consisted of two independent microfluidic circuits arranged on two levels, separated by a PET membrane. On the middle of the first, primary circuit, a primary human small intestinal model was inserted. The intestinal tissue was cultured in an integrated cell culture insert and provided a barrier function from the apical side of the intestine to the first circuit, allowing absorption. An on-chip micro-pump enabled the distribution from the basolateral side of the intestinal model to a liver equivalent, where potential substances could be metabolised. The microfluidic channel passed the bottom of the PET membrane, seeded with renal proximal tubule cells. This kidney model separated the first circuit from the second, excretory circuit. In this study, we combined this ADME approach with a skin biopsy to analyse toxicity, but this organ culture could also be replaced by any other organ equivalent, like neuronal tissue, lung tissue or others, depending on the target organ for toxicity. The combination of the four organs was cultured for up to 28 days and results showed a steady consumption of glucose and low LDH profiles during the complete culture period, providing evidence for a stable coexistence between the four tissues. The constitutive phase I and II enzymes were expressed in liver tissues and stayed constant on protein and mRNA level over the time cultured. The intestinal tissues expressed glucose transporters and its barrier function was proven by expression of tight junction proteins and stable, near to physiologic TEER values. Renal proximal tubule cells showed polarisation, a steady expression of tight junctions and metabolic activity. Thus, a new tool for subsystemic substance testing with a potential for ADMET profiling has been developed.

PS 2188 Development of an *In Vitro* Pharmacokinetic Model to Describe Nicotine Kinetics in a Multi-Organ Culture System

Y. Zhao[3], M. Yoon[3], M. D. Gaca[1], G. Phillips[1], H. Clewell[3], M. E. Andersen[3] and J. M. McKim[2]. [1]*British American Tobacco, R&D Centre, Southampton, United Kingdom;* [2]*IONTOX, Kalamazoo, MI and* [3]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

There has been a rapid growth in new *in vitro* methods designed to replace animal safety tests. Currently, there is a need for an *in vitro* integrated organ system coupled with an integrated "blood flow" that can examine systemic toxicity in humans. A well characterized system should allow for the development of pharmacokinetic models that predict drug or chemical movement *in vitro*. The aim of this study was to investigate the kinetics of nicotine in a two organ system as a case study of simulating inhalation exposure *in vitro* and to develop a pharmacokinetic model. A dynamic multi-organ plate (DMOP) comprised of a simple, two organ compartment system containing human lung tissue (MucilAir) in one chamber and human hepatocytes (HepG2) in the other. The chambers were linked by a fluidics/dialysis network. The dialysis membrane allows for exchange of test article and metabolites while maintaining optimized media conditions. A kinetic model was developed to describe the disposition of nicotine after a single concentration (1000 µM) was applied to the apical side of the MucilAir lung. The model included diffusion apical-basolateral, movement from basolateral lung-liver, perfusion of fluid through the dialysis tubing, and nicotine in the perfusate. Nicotine in the basolateral chamber diffused into the fluidics system via dialysis and was carried to the delivery well. A static sample from the basolateral and delivery chambers was collected every hour for 6 hrs. Perfusate from the fluidics system was collected at integrated one hour time intervals for 6 hr at a flow rate of 1 µL/min. Nicotine was first detected in the basolateral chamber at 1 hr and reached a maximum concentration (3-4 µg/mL) by 5 hr. Nicotine was first detected in the delivery chamber at approximately 3 hr and increased linearly up to 0.3 µg/mL. Nicotine in the run through perfusate was also detected at 3 hr. The current model simulated the observed concentration-time profiles

of nicotine in the DMOP system. Simulation results indicated that the test compound was successfully delivered from the exposure site to the target tissue through dialysis suggesting the applicability of the DMOP for simulating systemic effects of a compound after inhalation exposure with further optimization of flow patterns in the plate.

2189 Comparison of Ocular Irritancy Estimation Between BCOP and Reconstructed Human Cornea Like Epithelium (RhCE)

L. Milchak, D. Brandwein, J. Warmka and A. Stieffenhofer. *Medical Department, 3M Company, St Paul, MN.*

The 3M Strategic Toxicology Laboratory (STL) is an internal corporate resource providing support to 3M businesses that emphasizes the use of *in vitro* methodology. The STL routinely uses EpiOcular™, a three dimensional Reconstructed human Cornea like Epithelium (RhCE) model recently validated for identification of chemicals that do not require GHS classification for eye irritation or serious eye damage in OECD 492. A limitation of OECD 492 is that it does not allow differentiation between GHS Category 1 or GHS Category 2/2A. A commercially available *in vitro* ocular irritation assay that could allow that differentiation would be highly valuable. Although not included in the standard OECD 492 assay, EpiOcular™ is being examined to allow differentiation of GHS categories via an ET50 protocol which provides information on the degree of irritation and would also potentially allow the classification of corrosive materials. The Bovine Corneal Opacity test (BCOP) can allow that differentiation, but is not an easily adoptable method into a laboratory without a readily available, local source of bovine corneal tissue for routine use. The STL recently performed a comparison of the EpiOcular™ ET50 approach with historical BCOP test data from a variety of both water soluble and insoluble mixtures to examine the potential correlation between the approaches. The results showed that the best correlation was seen with more severe irritants, as the RhCE matched the BCOP corrosive classifications consistently. Mixtures that were non, mild, or moderate irritants in BCOP were generally found to be more irritating in the ET50 assay, suggesting that the RhCE may be more sensitive that BCOP. Non-water soluble mixtures were not able to be effectively removed from the RhCE, likely increasing the irritant response due to longer exposure times and necessitating experimentation with non-polar vehicles and rinsing solutions. In summary, further studies are needed to customize the ET50 approach in RhCE and better define the correlation with BCOP. The ET50 RhCE model may have limitations due to solubility of the test article and the ability to correlate with BCOP for less irritating test articles. In addition, novel methods to deal with non-polar substances will be needed to fully utilize the RhCE model, but the RhCE showed potential for correctly classifying ocular corrosives.

2190 Risk Assessment and Classification of Chemicals for their Irritation/Corrosive Potential Using an *In Vitro* Method of Bovine Corneal Opacity and Permeability Test (BCOP)

M. V. Patel[2], R. Verma[2], V. Dalal[2], S. Patel[2] and V. J. Piccirillo[1]. *[1]VJP Consulting, Inc., Ashburn, VA and [2]Toxicology, Jai Research Foundation, Valvada, India.* Sponsor: V. Piccirillo.

In vitro BCOP test is rapidly gaining popularity due to short term maintenance of the test system, economical to conduct, reliable predictive data; replacing the use of animals. This test was evaluated and confirmed for its sensitivity to identify chemicals inducing severe eye damage and chemicals not classified for eye irritation. BCOP test method is recommended as an initial step within a testing strategy such as the Top-Down approach. In compliance with TG OECD 437, solid materials were tested as 20% dilutions prepared in 0.9% NaCl solution for 4h at approximately 32°C followed by wash to remove test item. Liquid test item (undiluted) were applied for 10 minutes on corneas. Corneas were washed followed by incubation for 2h at approximately 32°C. Change in corneal opacity was determined by the quantum of light passing through the cornea using Opacitometer. Impairment of the corneal barrier function was measured by the amount of fluorescence passed through the cornea. Using the method of IVIS Score different chemicals were tested for their eye irritancy. IVIS score for Benzalkonium chloride, Chlorhexidine, Dibenzoyl-L-tartaric acid, Imidazole, Trichloroacetic acid, Ethanol and Dimethylformamide was found to be >55 and were classified under Category 1. 2,6-Dichlorobenzoyl chloride and Ethyl-2-methylacetoacetate were classified under No accurate/Reliable prediction as the IVIS was in between 3 and 55. Observation of test, using EDTA, di-potassium salt, Tween 20, 2-Mercaptopyrimidine, Phenylbutazone and Polyoxyethylene 23 lauryl ether showed the IVAS below 3 and hence categorised as Not Classified. Histopathology of Imidazole and Dimethylformamide revealed sloughing of surface squamous layer and stroma thicker. Hence, it is concluded that the chemicals used in this study have different intensity of eye damage. It is identified and classified efficiently by BCOP test procedures in this experiment.

2191 Evaluation of Topkat, Toxtree and Derek Ocular Irritation Qsar Models and Development of a Knowledge-Based Framework to Improve Severe Irritancy Predictions

B. Bhhatarai, D. M. Wilson, A. K. Parks and E. W. Carney. *TERC, The Dow Chemical Company, Midland, MI.*

Three commercially available QSAR models for ocular irritation - TOPKAT, ToxTree and Derek Nexus, were evaluated for their performance by using 1644 in-house and 123 ECETOC (European Centre for Toxicology and Ecotoxicology of Chemicals) compounds with existing *in vivo/ in vitro* ocular irritation data over all irritancy classes. Overall, the QSAR models performed poorly for all classes. However, the best consensus predictions for severe ocular irritants was 52% and 61.5%, for in-house and ECETOC compounds, respectively. The prediction performance was evaluated further by designing a knowledge-based chemical profiling framework that incorporates physico-chemical properties and electrophilic protein binding mechanisms. The utility of the framework was established by applying it to three additional publicly available ocular irritation datasets from ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) report, FDA-CFSAN (Food and Drug Administration-Center for Food Safety and Applied Nutrition) data and ECHA (European Chemical Agency) eChemportal data. By pre-filtering based on AlogP_MR (hydrophobicity with molar refractivity), the prediction for severe ocular irritants was improved to 73–77% for all studied datasets. The predictivity increased to 74–80% for compounds capable of undergoing harder electrophilic reactions, such as Schiff's base formation and acylation. This research stresses the need for reliable ocular irritation QSAR models based on mechanism-of-action and individual structural class. It also highlights the potential application of profiling compounds for chemical reactivity and physico-chemical properties to predict ocular irritancy.

2192 Is There Hope to Correctly Classify Severe Ocular Irritant Agrochemical Formulations Using *In Vitro* Methods: A Proof of Concept Using the Isolated Chicken Eye Test, Two Modified BCOP Protocols and an Epiocular™ ET50 Protocol

S. N. Kolle, B. van Ravenzwaay and R. Landsiedel. *Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

While some *in vitro* methods addressing ocular irritancy have gained regulatory acceptance, to date the Draize rabbit eye test (OECD TG 405) is the only world-wide regulatory accepted test for the determination of the full range of eye irritation potential. Although several *in vitro* methods for the severe eye irritation have gained regulatory acceptance, agrochemical formulations are nor explicitly included nor excluded from the applicability domain to predict severe ocular irritant formulations. Systematic analyses are only available for e.g. the hen's egg test- chorioallantoic membrane (HET-CAM), and bovine corneal opacity and permeability (BCOP, OECD TG 437) assays both showing that the used protocols do not provide sufficient sensitivity to reliably predict severe ocular irritating formulations. The purpose of this study was to evaluate whether the regulatory accepted isolated chicken eye (ICE, OECD TG 438) test including corneal histopathology (as suggested for evaluation of the depth of injury), as well two modified protocols of the BCOP and/ or an ET50 (exposure time reducing viability of treated tissue to 50%) protocol using the reconstructed cornea model EpiOcular could be used to predict severe ocular irritant agrochemical formulations. This proof of concept comprised the testing of ten to twelve agrochemical formulations with available *in vivo* data in each assay. In summary, based on the ICE evaluation described in OECD TG 438, one of the five severe ocular irritant formulations (UN GHS Cat 1) was predicted correctly. Using both modified protocol versions of the BCOP one of the four tested UN GHS Cat 1 formulations resulted in an IVIS just above the UN GHS Cat 1 classification border each. Lastly and most promising, the EpiOcular ET50 predicted four of five tested UN GHS formulations correctly with the fifths being close to the classification border. References: Kolle, Susanne N., et al. "The EpiOcular Eye Irritation Test is the Method of Choice for the *In Vitro* Eye Irritation Testing of Agrochemical Formulations: Correlation Analysis of EpiOcular Eye Irritation Test and BCOP Test Data According to the UN GHS, US EPA and Brazil ANVISA Classification Schemes." Alternatives to laboratory animals: ATLA 43.3 (2015): 181-198.

2193 Predictive Performance of the Short Time Exposure (STE) Test for Identifying Eye Irritation Potential of Chemical Mixtures

T. Abo[1], K. Saito[1], M. Miyazawa[1], Y. Nukada[1], K. Ei[1], J. Avalos[2] and H. Sakaguchi[1]. *[1]Kao Corporation, Tochigi, Japan and [2]Kao USA Inc., Cincinnati, OH.*

The Short Time Exposure (STE) test is an *in vitro* alternative eye irritation test based on the cytotoxicity of substances in SIRC cells (rabbit corneal cell line) following a five minute treatment. The STE test has been accepted as test guideline TG 491 by the Organisation for Economic Co-operation and Development (OECD) and the test assigns hazard classification and labeling of chemicals that induce serious eye damage as well as those that do not require classification for either serious eye damage or eye irritation, as defined by the United Nations (UN) Globally Harmonized System of Classification and Labeling of Chemicals (GHS) in a tiered testing strategy (i.e., bottom-up or top-down approach) with alternative test methods. This study evaluated the predictive performance of the STE test to identify UN GHS No Category and other irritant categories (i.e., GHS Category 1 and 2) for 40 chemical mixtures that include surfactants in base formulations for personal care products, and fabric and home care products. The STE test correctly identified 30 tested mixtures classified as GHS irritant categories and 5 out of 10 tested mixtures classified as GHS Not Classified. The sensitivity, specificity, positive predictivity, negative predictivity, and overall accuracy of the STE test were 100% (30/30), 50% (5/10), 86% (25/30), 100% (5/5), and 88% (35/40), respectively. These predictive performances were comparable to or greater than those in other *in vitro* eye irritation tests that have been accepted as an OECD test guideline like BCOP (TG 437) and ICE (TG 438). This suggests that the STE test has sufficient predictivity for identifying the eye irritation potential of chemical mixtures. Since no false negatives in this study were found, this indicates that the STE test may be incorporated into a bottom-up approach.

2194 Correlation of Two *In Vitro* EpiOcular Test Methods and Consumer Eye Irritation Data for Cleaning Products

L. A. Strazdas[3], K. E. Page[3], P. D. Elias[3], N. Wilt[1], G. Mun[1], E. Sly[1], N. Sadowski[2], J. Saudan[3] and K. Mainquist[3]. *[1]Institute for In Vitro Sciences, Inc., Gaithersburg, MD; [2]Institute for In Vitro Sciences, Inc., Gaithersburg, CA and [3]The Clorox Company, Pleasanton, CA.*

The Clorox Company has used the EPA EpiOcular assay (EO) to predict ocular irritancy potential of cleaning products without the use of animals. The US EPA - Office of Pesticide Programs has accepted EO data in replacement of animal tests for ocular irritancy of cleaning products with antimicrobial claims. Another assay utilizing EpiOcular tissue, the Eye Irritation Test (EIT), was recently accepted by OECD (TG 492) for classifying materials that do not require ocular irritation labelling under GHS. EO utilizes multiple exposure times to calculate the test material exposure time to cause 50% viability (ET50). EIT also evaluates ocular irritation, using mean relative viability (MV30) after single exposure time (30 minutes) and post exposure incubation. Ten reference materials with known irritancy levels were tested in EO and EIT. In the EIT, 5 materials resulted in MV30 of >60% with GHS "No Category" classification. The remaining 5 test materials with MV30 <60% were classified as ocular irritants. In the EO, two test materials were predicted to be EPA Category IV (ET50 > 70min), 5 were predicted to be Category III (70 min > ET50 >4 min), and 3 were predicted to be Category I (ET50 < 4 min).The results of both EIT and EO assays gave similar rankings in level of irritancy. For EO, both ET50 values and estimated viability values after 30 minute of exposure to test materials (same exposure time as EIT) were compared to EIT results. Correlation of the EO and EIT MV30 scores resulted in an r2 value of 0.91, adding strength to the observed correlation of the methods. The collected human eye irritation data further supported this case, aligning with predicted irritation of the tested materials.

2195 An Approach to Skin Sensitization Quantitative Risk Assessment Using a Bayesian Integrated Testing Strategy to Derive Potency

C. Ryan[2], J. Jaworska[3], P. Kern[1] and G. F. Gerberick[2]. *[1]The Procter & Gamble Company, Beijing, China; [2]The Procter & Gamble Company, Mason, OH and [3]The Procter & Gamble Company, Strombeek-Bever, Belgium.*

An exposure-based quantitative risk assessment (QRA) process for skin sensitization can be conducted to determine safe use levels of ingredients with sensitization potential in consumer product. The foundation of a QRA is the identification of a No Expected Sensitization Induction Level (NESIL), analogous to a NOAEL in general toxicology, for the chemical of interest. The NESIL is typically derived from animal (e.g. Local Lymph Node Assay (LLNA)) and human data by a weight of evidence approach, taking into consideration all relevant data. However, nowadays the risk assessment must often rely on non-animal methods as animal tests are no longer the first method of choice or not feasible at all, e.g. for cosmetic ingredients. Use of a Bayesian Network Integrated Testing Strategy (BN-ITS) was explored as a method to derive NESILs for use in QRA. The BN-ITS structure represents the underlying mechanistic processes described in the skin sensitization Adverse Outcome Pathway and integrates data from three validated methods, along with bioavailability and chemistry inputs. Data from the Direct Peptide Reactivity Assay represents Key Event (KE) 1, covalent binding to skin proteins. KE 2, the keratinocyte inflammatory response, is covered by the KeratinoSens® assay. Data input for KE 3, activation of dendritic cells, is provided by the Human Cell Line Activation Test. The BN-ITS expresses skin sensitization potency in the LLNA as a probability distribution across 4 potency classes; non-sensitizing, weak, moderate and strong. The uncertainty of the potency prediction is assessed by conversion of the probability-based predictions to Bayes factors (BF). Only potency class predictions with BF indicating substantial evidence are considered. To apply this output for use in a QRA, a conservative default value is assigned to each class for use as the NESIL. This approach represents the first step in progressing the use of non-animal methods from simple hazard identification to use in risk assessment.

2196 Bayesian Integrated Testing Strategy (ITS) for Skin Sensitization Potency Assessment - A Decision Support System for Quantitative Weight of Evidence and Adaptive Testing Strategy

J. Jaworska[5], A. Natsch[1], C. Ryan[4], J. Strickland[2], T. Ashikaga[3] and M. Miyazawa[6]. *[1]Givaudan Schweiz AG, Dübendorf, Switzerland; [2]ILS/NICEATM, Research Triangle Park, NC; [3]Shisheido Company Limited, Kanagawa, Japan; [4]The Procter & Gamble Company, Mason, OH; [5]The Procter & Gamble Company, Strombeek-Bever, Belgium and [6]R&D Safety Science Research, Kao Corporation, Tochigi, Japan.*

The present Integrated Testing Strategy (ITS-3) for skin sensitization potency assessment builds upon previous work using a Bayesian network (BN) framework. ITS-3 was constructed with the following objectives: 1) to improve precision and accuracy beyond ITS-2 by better integration of chemistry and biology and refinement of the bioavailability inputs; 2) to consider the applicability domains of individual assays in the prediction process; 3) to replace the U937 test for dendritic cell activation with the h-CLAT. We show how incorporation of the novel components reduced the uncertainty of predictions of all potency classes compared with ITS-2. This is the first ITS incorporating quantitative data from three validated alternative assays. The adverse outcome pathway structure (i.e. sequence of key events) related to the first three key events, are encoded in the BN ITS-3 structure. Novel to ITS, bioavailability is applied to both *in vivo* and *in vitro* data. The prediction is provided as the pEC3 probability distribution over four potency classes. The probability distribution, which quantifies prediction uncertainty, is converted to Bayes factors to: 1) remove prediction bias introduced by the training set class distribution; 2) express uncertainty, allowing transparent and consistent criteria to accept a prediction. A novel database of 207 chemicals with a full set of *in vivo* and *in vitro* data was developed. The accuracy of the ITS-3 on the external test set (n=60) was: hazard - 100%, GHS potency classification - 96%, potency (four classes) - 89%. Practical case studies illustrate that a mechanistically interpretable testing strategy can be developed for every chemical.

2197 Prediction of Systemic Bioavailability Using *In Vitro* Skin Absorption and Epidermal and Hepatic Metabolism of Phosphate Alkyl Ethoxylate Esters

J. D. Manwaring[3], T. Kirsch[2], C. M. Obringer[3], D. Foltz[3], T. Baker[3], C. Goebel[2] and J. A. Troutman[3]. *[1]Procter & Gamble, Mason, OH; [2]Procter & Gamble, Schwalbach, Germany and [3]Global Product Stewardship, Procter & Gamble, Mason, OH.* Sponsor: G. Daston.

Approaches to assess the role of absorption, metabolism and elimination (AME) of cosmetic ingredients that are based on the integration of different *in vitro* data are important for their safety assessment specifically due to the ban on animal testing in the European Union. In order to estimate systemic exposure (AUC) to phosphate alkyl ethoxylates (mono- and di-esters) *in vitro* following typical product application conditions, the bioavailable fraction was determined from skin penetration estimations and subsequent epidermal and systemic metabolic hydrolysis of the parent compound assessed in human skin S9 and human liver S9 fractions. First, conservative skin penetration estimates and skin S9 hydrolysis data were used to estimate the amount of the individual ester molecule that reached the general circulation unchanged by applying the following pharmacokinetically relevant parameters: a) *in vitro* intrinsic clearance (CLint) kinetics to quantify the epidermal metabolism; b) the estimated human skin S9 protein density in the viable epidermis; c) the skin permeability coefficient; d) the calculated Mean Residence Time (MRT) in the viable epidermis and e) the viable epidermis thickness. The resulting estimated internal doses of the remaining individual non metabolized ester compounds available following skin passage were in the range of up to 10 percent of the applied dose. In the next step, *in vitro* human liver S9 CLint values (451 and 99 µL/min/mg S9 protein, respectively, for the mono- and di-ester) and whole liver mass and S9 density were used to calculate the scaled hepatic intrinsic clearance. Using the well-stirred model hepatic blood clearance was calculated and used as a conservative estimate of the total clearance, which then allowed extrapolation of the systemic AUC using internal dose and hepatic blood clearance. Comparison to an AUC similarly deduced from a historical rat NOEL from a repeated dose toxicity study indicates that appropriate toxicokinetic information and relative exposure estimates can be generated based solely on *in vitro* data.

2198 Intra/inter Reproducibility Analysis of the *In Vitro* Skin Sensitization Assay Using Reconstructed Human Epidermis

K. Saito[2], H. Mizumachi[2], M. Miyazawa[2], M. Ikezumi[1], A. Umetsu[1], H. Motohashi[1], M. Sakuma[3], M. Takeyoshi[3] and N. Imai[3]. *[1]Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan; [2]Kao corporation, R&D safety Science Research, Tochigi, Japan and [3]KOSÉ Corporation, Fundamental Research Laboratories, Tokyo, Japan.* Sponsor: J. Avalos.

Background and Purposes: To appropriately evaluate lipophilic chemicals that are difficult to be evaluated by existing *in vitro* skin sensitization assays, a novel *in vitro* skin sensitization assay uses reconstructed human epidermis to evaluate the sensitizing potential of chemicals based on the expression of multiple marker genes relevant to keratinocyte responses (inflammatory response; ATF3 and IL-8, cytoprotective response; DNAJB4 and GCLM). In this assay, test chemicals dissolved in lipophilic vehicles were applied directly on the tissue. Previous studies showed that this assay had good predictive performance of sensitizing potential for various chemicals including lipophilic substances (SOT 2015). In this study, three laboratories (Kao, KOSÉ, & Food and Drug Safety Center) assessed the transferability and intra/inter reproducibility of the assay as an initial step toward the establishment of this test method. Materials and methods: The transferability and intra/inter-laboratory reproducibility of the assay were evaluated using three non-coded chemicals (two positive control chemicals and one negative control chemical) in the transferability test and three coded chemicals (two sensitizers and a non-sensitizer) in the intra/inter-laboratory reproducibility test. Each chemical was applied to the tissue model. After 6 hours, the expression of four marker genes (ATF3, IL-8, DNAJB4 and GCLM) was quantified. A tested chemical was judged as a positive when the expression of at least one marker gene of the four exceeded the cut-off value. Results and discussions: In the transferability study, all three laboratories predicted the positive/negative outcomes for the three chemicals. In the intra/inter-laboratory study, the complete concordance with the *in vivo* data for three tested chemicals was reproducible in three laboratories. These data suggested that this assay could predict the skin sensitization potential of chemicals with good intra/inter laboratory reproducibility.

2199 Evaluation of the Episkin RHE Model in the SENS-IS Assay for Prediction of Skin Sensitization of Chemicals

F. Cottrez[2], E. Boitel[2], C. Pellevoisin[1], N. Seyler[1] and H. Groux[2]. *[1]EPISKIN Academy, Lyon, France and [2]ImmunoSearch, Grasse, France.* Sponsor: E. Dufour.

While *in vitro* method for acute local toxicological endpoints such as skin corrosion/irritation are now widely used to replace animal tests, more complex endpoints such as skin sensitization are more difficult to manage. Based on a comprehensive analysis of skin sensitization, the SENS-IS assay uses the quantitative analysis of multiple specific biomarkers expressed in EPISKIN model a 3D reconstructed human epidermis. Immunosearch, developed this assay after analyzing genes modulated during the sensitization process either on mice (LLNA) or human (blisters). The panel of sensitization biomarkers defined includes genes already identified as markers, such as the ARE family, and others not yet associated with the sensitization process (called SENS-IS gene subset). The objective of the study was to evaluate the impact of using the Episkin-RHE model instead of the EPISKIN model in the SENS-IS assay. The Episkin-RHE is reconstructed from young male donor keratinocytes rather than adult female cells, in a chemically defined medium without serum and has 0.5 cm2 surface compared to 1.07 for the EPISKIN model. For this, a set of 4 reference chemicals, 2 sensitizers, the 2,4,6-TriNitroBenzene sulfonic acid (TNBS) and the Hexyl cinnamaldehyde (HCA) and 2 non-sentizers, the dimethyl sulfoxide (DMSO) and the sodium laureth sulfate (SLS) have been evaluated. Results of three different runs showed that the SENS-IS assay using the Episkin-RHE model correctly classified the 4 molecules for irritation and sensitization. The SLS is classified as irritant and non sensitizer, TNBS (1%) is non irritant and sensitizer, HCA is irritant and sensitizer and DMSO slighty irritant and non sensitizer. Although some adjustments were made : total volume used to adjust to the surface size difference, threshold for positivity to adjust to the background level, Within Laboratory Reproducibility was as good as the one measured with the EPISKIN model (>95%) These preliminary results show that the Episkin-RHE model could be used in the SENS-IS assay to assess the sensitizing potential of chemicals. Further studies are engaged with using a more comprehensive set of molecules. If confirmed, these results will allow toxicologists, familiar with either the EPISKIN or the Episkin-RHE model to assess both skin irritation according to OECD TG 439 and skin sensitization with the SENS-IS assay with the same model.

2200 Identification of Pre- and Pro-Haptens for Skin Sensization with Non-Animal Test Methods

D. Urbisch[3], E. Fabian[3], N. Honarvar[3], S. N. Kolle[3], A. Mehling[1], T. Ramirez[3], D. Vogel[3], W. Teubner[2] and R. Landsiedel[3]. *[1]BASF Personal Care and Nutrition, Düsseldorf, Germany; [2]Experimental Toxicology and Ecology, BASF Schweiz AG, Basel, Switzerland and [3]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

Since pro-haptens may be metabolically activated in the skin, information on xenobiotic metabolizing enzyme activities in cell lines used for testing of sensitization *in vitro* is of special interest. The metabolic activity of e.g. N-acetyltransferase 1 and esterase in the keratinocyte (KeratinoSens™ and LuSens) and dendritic cell-like cells (THP-1) was previously demonstrated and aldehyde dehydrogenase activities were found in KeratinoSens™ and LuSens cells. Activities of the investigated cytochrome P450-dependent alkylresorufin O-dealkylases, flavin-containing monooxygenase, alcohol dehydrogenase as well as UDP glucuronosyl transferase activities were below detection in all investigated cell lines. A set of 32 putative pre- and pro-haptens (no obvious structural alert for peptide reactivity but positive *in vivo*) was routinely tested using the above mentioned cell lines as well as in the in chemico direct peptide reactivity assay (DPRA). 18 of the compounds were unexpectedly positive in the DPRA und further analyzed by LC/MS techniques to clarify the reaction mechanism leading to true positive results in this assay. Oxidation products like dipeptide formations or the oxidation of the peptide-based sulfhydryl group led to positive results for benzo[a] pyren or 5-amino-2-methylphenol, respectively. In contrast, covalent peptide adducts were identified for 12 putative pre-haptens, indicating the DPRA to be suitable for compounds requiring abiotic oxidation to get activated. For some DPRA negatives, the keratinocyte and dendritic cell based assays provided true positive results. A combination of DPRA, KeratinoSens™ and h-CLAT within a '2 out of 3' prediction model provided a high sensitivity of 81% for the set of pre-/pro-haptens. The sensitivity in a set of 119 direct haptens was comparable (sensitivity =

82% when compared to LLNA). Fabian, E., et al. "Xenobiotic metabolizing enzyme activities in cells used for testing skin sensitization *in vitro*." Archives of toxicology 87.9 (2013): 1683-1696. Jäckh, Christine, et al. "Relevance of xenobiotic enzymes in human skin *in vitro* models to activate pro-sensitizers." Journal of Immunotoxicology 9.4 (2012): 426-438.

PS 2201 The Potential of Protein Reactivity to Predict Skin Sensitizing Potency: Of Peptide Depletion, Reaction Time and Tested Concentrations

B. Wareing[2], S. N. Kolle[2], D. Urbisch[2], D. Mulliner[2], B. van Ravenzwaay[2] and R. Landsiedel[2]. [1]*Computational Chemistry and Biology, BASF SE, Ludwigshafen am Rhein, Germany and* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

Several non-animal test methods addressing key events in the sensitization process have passed formal validation and OECD (draft) test guidelines are available. One of these methods is the direct peptide reactivity assay (DPRA) assessing the ability of a chemical to bind to proteins to form a complete antigen (OECD TG 442C). The test is used to obtain a yes/no answer on whether the substance has a protein-binding potential. For a complete risk assessment, however, an estimation of a chemical's potency is also needed. In this study we examined if an assessment of potency could be achieved by 1) determining reactivity class cut offs based on published data on 199 substances for the DPRA performed according to OECD 442C to predict UN GHS sensitizer classes, 2) a variant of the DPRA assessing reaction kinetics (time and concentration) for 12 substances or 3) an extended protocol testing several test substance concentrations for 50 reference substances and estimating the concentration of a test substance that is needed to cause a peptide depletion of 6.38% (EC6.38%). Results of the first approach indicated that cut offs to differentiate the UN GHS sensitizer classes 1A and 1B could indeed be defined. Secondly, evaluating the reaction time based assay in which several time points between 5 min and 24 hours were assessed, it was found that not all reactions followed ideal kinetics. Hence further investigations are needed to eventually derive a reaction time based prediction model. The results of the 3rd approach (the standard protocol of the DPRA was amended by testing three concentrations i.e. 1, 10, and 100 mM) indicated that potency classes could be assigned using the EC6.38% value to assess potency. In summary, using quantitative information derived from the DPRA in particular using EC6.38% value may support the assessment of the skin sensitizing potency. Reference: Urbisch, Daniel, et al. "Assessing skin sensitization hazard in mice and men using non-animal test methods." Regulatory Toxicology and Pharmacology 71.2 (2015): 337-351.

PS 2202 Multivariate Models for Prediction of Human Skin Sensitization Hazard

J. Strickland[6], Q. Zang[6], M. Paris[6], D. M. Lehmann[2], N. Kleinstreuer[6], D. Allen[6], N. Choksi[6], J. Matheson[1], A. Jacobs[4], A. Lowit[3] and W. Casey[6]. [1]*CPSC, Rockville, MD;* [2]*US EPA/ORD/NHEERL, Durham, NC;* [3]*US EPA/OSCSPP/OPP, Washington, DC;* [4]*US FDA/CDER, Silver Springs, MD;* [5]*NIEHS/NICEATM, Durham, NC and* [6]*ILS, Inc., NIEHS/NICEATM, Durham, NC.*

One of ICCVAM's highest priorities is the development and evaluation of non-animal approaches to identify potential skin sensitizers. The complexity of biological events necessary for a substance to elicit a skin sensitization reaction suggests that no single alternative method will replace the currently accepted animal tests. ICCVAM is evaluating machine learning approaches to integrate relevant data based on the OECD adverse outcome pathway for skin sensitization to predict human skin sensitization hazard. We obtained data on 96 chemicals from the direct peptide reactivity assay (DPRA), human cell line activation test (h-CLAT), KeratinoSens assay, six physicochemical properties, and an *in silico* read-across prediction of skin sensitization hazard. These data were used as inputs for two machine learning approaches to predict human skin sensitization hazard, support vector machine and logistic regression, which were each applied to 12 different combinations of the input variables. Models were trained on a set of 72 substances and tested on an external set of 24 substances. The input variable set containing DPRA, h-CLAT, KeratinoSens, read-across, and log P data performed the best for both approaches: accuracy = 99% (71/72), sensitivity = 98% (50/51), and specificity = 100% (21/21) for the training set; and accuracy = 96% (23/24), sensitivity = 93% (14/15), and specificity = 100% (9/9) for the test set. This integrated approach predicted human skin sensitization hazard better than the local lymph node assay or any in chemico, *in vitro*, or *in silico* method alone. These results suggest that computational methods are promising tools to effectively identify potential skin sensitizers without animal testing. This abstract does not represent US EPA policy. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

PS 2203 Reducing False Negatives of Chemicals in the *In Vitro* Skin Sensitization Test

K. Narita[2], P. T. Vo[2], F. Nakagawa[2], H. Kojima[1] and H. Itagaki[2]. [1]*National Institute of Health Sciences, Tokyo, Japan and* [2]*Yokohama National University, Yokohama, Japan.*

Difficulties in accurately assessing skin sensitization induced by phthalic anhydride using *in vitro* assays are well documented. We reported that THP-1 cells exposed to phthalic anhydride in liquid paraffin (LP) vehicle cells exhibited significant release of Interleukin 8 (IL-8).1 The aim of this investigation is to elucidate the exposure mechanisms for application to h-CLAT. THP-1 cells were exposed for five minutes to NiSO4, phthalic anhydride and acid suspended in LP, then washed and cultivated for 24 hours. We measured viability and IL-8 release as well as CD86 and CD54 expression of the THP-1 cells using a WST-8 assay in accordance with ELISA and the h-CLAT measurement protocol. Also, we measured sequentially the absorbance of phthalic anhydride contained in an LP/water binary-phase vehicle. As a result, the IC50 for phthalic anhydride was 1,315 ug/ml in aqueous solution after a 24-hour exposure, and that for phthalic acid was 1,305 ug/ml. These results are very similar. But, in the LP vehicle after a five-minute exposure, the IC50 for phthalic anhydride was 736 ug/ml, which was a mere half of the 1,421 ug/ml for phthalic acid. Also, whereas phthalic anhydride was found to have released IL-8 after exposure in the LP vehicle, phthalic acid did not. Results of using the h-CLAT in aqueous solution assessed phthalic anhydride as negative, but using the LP vehicle exposure method, phthalic anhydride exhibited augmentation in CD54 expression of the THP-1 cells. In addition, using either aqueous or LP vehicle, NiSO4 was found to have augmented CD54 expression on THP-1 cells. On the other hand, the results of a quantitative analysis of phthalic anhydride showed that the phthalic anhydride was present for at least 5 minutes in the aqueous phase because of the sustained-release from the LP. In conclusion, we found that LP vehicle exposure is likely to enable proper evaluation of the sensitization by phthalic anhydride. Future investigation will use this technique to examine a wide range of false-negative chemicals, including other acid anhydrides and water-insoluble chemicals. 1. K. Narita et al., 2014, 9th World Congress on Alternatives and Animal Use in the Life Sciences proceedings, 3(1), 119.

PS 2204 Development of Photo-Direct Peptide Reactivity Assay

H. Nishida[1], T. Ashikaga[1], M. Hirota[1], S. Onoue[2], Y. Seto[2] and H. Kouzuki[1]. [1]*Shiseido Research Center, Yokohama-shi, Japan and* [2]*Department of Pharmacokinetics and Pharmacodynamics, University of Shizuoka, Shizuoka-shi, Japan.*

Several *in vitro* skin sensitization tests corresponding Adverse Outcome Pathway (AOP) have been developed. The Direct Peptide Reactivity Assay (DPRA) is a test which evaluates protein reactivity of test chemicals by using model peptides containing cysteine or lysine. The human cell line activation test (h-CLAT) is detecting activation of dendritic cell exposed by skin sensitizers. The KeratinoSens™ is an assay monitored keratinocyte response induced by skin sensitizers. While photo-h-CLAT and photo-KerainoSens™ were developed, study on photo-DPRA has not been reported. The purpose of the present study is to establish a photo-DPRA. The Photo-DPRA was developed based on the DPRA Standard Operating Procedure (SOP). Simulated sun light irradiation process was added to the DPRA SOP. In order to optimize an irradiation condition, several irradiation levels (0-36J) were tested. As a result, 21.6J was selected as the appropriate amount. More than 10 non photosensitizers such as cinnamaldehyde, 4-methylbenzylidene camphor and phenytoin did not enhance peptide depletion in the presence of irradiation. Regarding more than 20 photosensitizers tested in the study such as 3,3',4',5-tetrachloro salicylanilide, 6-methylcoumarin, ketoprofen and musk ambrette, the levels of peptide depletion in the presence of irradiation was significantly enhanced compared with the non-irradiation condition. Interestingly, all photosensitizers tested in this study reacted with cysteine peptide, while only few photosensitizers react with lysine peptide. Positive predictivity of photo-DPRA for photosensitizers was approximately 90% (isoniazide and indomethacin were judged as negative). These results suggested that the photo-DPRA would be useful to distinguish photosensitizer from both sensitizer and non sensitizer. A combination of non-animal methods including the photo-DPRA based on Integrated Testing Strategy (ITS) will be needed to fully substitute for the animal tests.

2205 Prediction of Skin Sensitizing Potency of Chemicals by the Alternative Cell-Based Assay GARD

T. Lindberg, K. Zeller, A. Forreryd, A. Chawade and M. Lindstedt. *Department of Immunotechnology, Lund University, Lund, Sweden.*

Allergic contact dermatitis (ACD) is a public health concern with a prevalence of 20 % among the European population. It is caused by low-molecular weight chemicals, which induce a hypersensitivity reaction in the affected individual. As exposure to chemicals in everyday life increases, reliable test methods are needed for correct risk assessments. The Genomic Allergen Rapid Detection (GARD) assay is a cell-based model that uses a predictive biomarker signature to classify chemicals according to their sensitizing capacity. In the context of risk assessment, it is crucial to also determine the sensitizing potency of chemicals. We previously hypothesized that chemical mechanistic domains are linked to the sensitizing potency of a chemical and have demonstrated that cell-cycle-associated and metabolic pathways correlate with sensitizing potency. In this study, we have applied advanced bioinformatics approaches to identify a transcriptional biomarker signature, predictive of the sensitizing potency. The dataset encompasses microarray data from genome-wide transcriptional analyses using cell stimulations with >60 unique chemicals. In addition, we will present results from an in-house validation of the prediction model for potency, using a dataset including approximately 40 new chemicals with known potency and chemical mechanistic domains. The model is designed to predict potency according to CLP classifications (European Chemical Agency), which label the chemicals as either sensitizing (1A=strong; 1B=weak) or non-sensitizing (no category). To gain insights into the biological mechanisms involved, further investigations will aim to identify genes and/or pathways that correlate with potency. New *in vitro* methods for determining the sensitizing potency of chemicals are urgently needed in order to allow correct risk assessment of chemicals. GARD is a promising tool for classification of chemicals according to their skin sensitizing ability. Here, we also present GARD's ability to deliver high quality potency information in a novel data analysis strategy.

2206 Applicability Domain of the U-SENS Test Method for Skin Sensitization Testing over 175 Chemicals

C. Piroird, L. Nardelli, J. Cotovio and N. Alepee. *L'Oreal, Aulnay Sous Bois, France.* Sponsor: E. DuFour.

The U-SENS method is proposed to address the third key event (dendritic cell activation) of the skin sensitisation Adverse Outcome Pathways by quantifying changes in the expression of cell surface CD86 marker associated with the process of activation of DC, in the human myeloid cell line U937, following exposure to sensitisers. The measured expression levels of CD86 marker are used for supporting the discrimination between skin sensitisers and non-sensitisers. The aim of the current study was to define the applicability domain and the limitation of the test method. Over the 175 tested chemicals, the large applicability domain of U-SENS was demonstrated through the wide diversity of use categories and chemical reactivities. The predictive capacity was comprehensively explored by comparing results obtained to human and LLNA data of a total of 175 substances including both cosmetic and non-cosmetic ingredients (70:30). U-SENS showed 79% specificity, 90% sensitivity and 88% accuracy. When focusing on the false negative and false positive predictions, no specific class could be singled out or excluded from the applicability domain, the misclassified substances being assigned to different classes. Interestingly, the test method was able to correctly predict 8 chemicals characterized by a high octanol-water partition coefficient. Another source of false negative results was the possible color interference of dyes and fluorescence used in flow cytometry. However, the 14 dyes examined did not cause such interference, and were correctly identified as sensitizers. Furthermore, 11 substances reported to be pre-haptens or pro-haptens were all correctly predicted by U-SENS Therefore, despite technical limitations and the limited metabolic capacity of the U937 cell line, the test method can be useful to predict lipophilic compounds (when they gave positive outcomes) or dyes (when no color interference were observed or when they gave positive outcomes) and is able to detect some known pre/pro-haptens.

2207 Integrated Test Approach for *In Vitro* Skin Irritation and Sensitization Testing

H. Hofman-Huether and H. Gehrke. *In vitro Pharmacology and Toxicology, Eurofins BioPharma Product Testing Munich GmbH, Planegg, Germany.* Sponsor: A. Poth.

The use of non-animal test methods and the reduction of animals in toxicology research is one of the outmost concerns at Eurofins BioPharma Product Testing Munich GmbH. It was the purpose of this study to investigate whether a reasonable combination of *in vitro* methods can verify the results of the *in vivo* testing and whether such an approach can contribute to a reduction of animal testing and might be a reliable alternative testing strategy. A retrospective analysis of data from *in vivo* LLNA studies was performed and set against the results from parallel performed *in vitro* skin irritation and sensitization studies in order to elucidate their comparability. Both endpoints, the skin irritation and sensitization potential of the substances were also determined by different *in vitro* methods. Skin irritation was addressed using the commercial reconstructed 3D human skin model. In addition, the sensitizing potential of the substances was assessed by DPRA and h-CLAT. Results: A direct correlation of both systems in analyzed parameters could be derived. Each substance tested positive in the *in vitro* skin irritation test was confirmed by high irritation potential in the LLNA assay. In contrast, substances assessed to be irritating in the *in vivo* LLNA assay were not necessarily identified as such in the *in vitro* skin irritation test. Furthermore, substances exhibiting a sensitizing potential were successfully identified as skin sensitizers by the combination of *in vitro* assay. Despite the fact that both systems were performed using completely different set ups with respect to administration and material coming from different species (human versus mouse), the correlation of both systems was tremendous high. Conclusion: In conclusion, the results indicate that an *in vitro* testing strategy seems to be useful to predict irritation and sensitization. Especially in the event of irritating substances, the results of the LLNA can be challenging and can only be assessed at low doses due to interference of irritating and sensitizing effects. We could demonstrate that in this case a meaningful combination of *in vitro* methods offer a reliable alternative testing strategy and additionally contribute to a significant reduction of used animals.

2208 A Weight of Evidence Investigation of Agrochemical Actives and Formulations for Skin Sensitization Potential Using *In Silico, In Chemico, In Vitro*, and GHS Concentration Threshold Approaches

S. C. Gehen[1], R. Acosta Amado[1], N. R. Visconti[2], L. Luna[2], M. Corvaro[1], J. McFadden[2], D. M. Wilson[2] and R. S. Settivari[2]. [1]*Dow AgroSciences, Indianapolis, IN and* [2]*Dow Chemical Company, Midland, MI.*

Assessment for skin sensitization potential of chemicals is an important component of the safety evaluation process. The key biological events underlying the skin sensitization process have been outlined, facilitating a weight of evidence (WoE) analysis for this endpoint. Herein, we describe our application of *in silico* (DEREK and TIMES), Direct Peptide Reactivity Assay (DPRA), KeratinoSens assay and GHS concentration threshold (CT) approaches, for the evaluation of the skin sensitization of prospective agrochemical active ingredients (AIs) and formulations. Initially, the *in silico* analyses, KeratinoSens and DPRA were conducted on up to 20 AIs for which the *in vivo* sensitization data were already available. The *in silico* models correctly predicted all sensitizing AIs, however identified 10 out of 14 non-sensitizers as false positives relative to animal results. The KeratinoSens and DPRA had overall accuracies of 85% and 90%, respectively. A WoE (2 out of 3) approach comprising the *in silico*, the KeratinoSens and DPRA data correctly identified sensitization potential of all tested AIs compared to existing *in vivo* data. Next, the performance of the KeratinoSens, DPRA and CT analyses were evaluated on up to 16 agrochemical formulations containing a single AI or 9 formulations containing 2 or 3 AIs. The KeratinoSens and DPRA provided accuracies of 100% and 86%, respectively for identifying formulations containing a single AI. For formulations with multiple AIs, the KeratinoSens and DPRA had accuracies of 78% and 100%, respectively. The CT approach provided very good correspondence compared to *in vivo* data. A WoE (2 out of 3) approach comprising of the DPRA, KeratinoSens and CT approaches correctly predicted all tested agrochemical formulations compared to *in vivo* data. Taken together, these results provided evidence to support potential application of the above assays in a WoE approach for the evaluation of the skin sensitization potential of agrochemical AIs and formulations.

2209 Combining *In Silico* and *In Vitro* Methods to Improve the Accuracy of Skin Sensitization Predictions for Chemicals

M. Lamm, N. Sadowski and K. G. Norman. *Institute for In Vitro Sciences, Gaithersburg, MD.* Sponsor: H. Raabe.

Given the complex cascade of events leading to skin sensitization, it is generally thought that an integrated testing approach combining multiple assays and *in silico* tools will be needed to fully replace the animal based methods. Recently, regulatory guidelines were published for two non-animal skin sensitization testing methods, the ARE-Nrf2 Luciferase Test Method (also referred to as the KeratinoSens™ Assay) and the Direct Peptide Reactivity Assay (DPRA). The KeratinoSens™ assay is a cell-based reporter gene method which identifies skin sensitizers by measuring the induction of luciferase under the control of the antioxidant response element (ARE) derived from the human AKR1C2 gene. The DPRA is an *in chemico* assay which identifies skin sensitizers based on their reactivity with synthetic peptides containing either lysine or cysteine. In this study we sought assess the skin sensitization potential of a set of 18 chemicals, including chemicals requiring metabolic activation to cause skin sensitization, using *in silico* tools (OECD Toolbox) in combination with the DPRA method and the KeratinoSens™ assay. First, the chemicals were evaluated using the OECD Toolbox to identify structural alerts for each chemical and metabolites. If pro-haptens, chemicals which may cause skin sensitization following conversion into electrophilic molecules by skin enzymes, were identified by structural alerts then enzymatic steps using human liver microsomes were incorporated into the KeratinoSens™ and DPRA test methods to improve accuracy as the standard protocols for these assays generally do not correctly predict pro-haptens. For the six chemicals requiring activation to become sensitizing, including cinnamic alcohol, use of the standard protocol led to false negatives whereas when the enzymatic steps were added the chemicals were correctly classified. The results indicated that testing strategies based on structural alerts and modification of standard protocols when necessary may allow for improved accuracy of skin sensitization predictions.

2210 Agent-Based Computational Modeling of Cell Culture: Understanding Dosimetry *In Vitro* as Part of *In Vitro* to *In Vivo* Extrapolation

R. Conolly[2], W. Cheng[3], C. Eklund[2], J. Samet[1], P. Wages[1], K. Lavrich[1] and S. Bhattacharya[4]. *[1]US EPA, Chapel Hill, NC; [2]US EPA, Research Triangle Park, NC; [3]US EPA/ORISE Internship/Research Participation Program, Research Triangle Park, NC and [4]The Hamner Institutes, Research Triangle Park, NC.*

Quantitative characterization of cellular dose *in vitro* is needed for alignment of doses *in vitro* and *in vivo*. We used the agent-based software, CompuCell3D (CC3D), to provide a stochastic description of cell growth in culture. The model was configured so that isolated cells assumed a "fried egg shape" but became increasingly cuboidal with increasing confluency. The surface area presented by each cell to the overlying medium varies from cell-to-cell and is a determinant of diffusional flux of toxicant from the medium into the cell. Thus, dose varies among cells for a given concentration of toxicant in the medium. Computer code describing diffusion of H2O2 from medium into each cell and clearance of H2O2 was calibrated against H2O2 time-course data (25, 50, or 75 uM H2O2 for 60 min) obtained with the Amplex Red assay for the medium and the H2O2-sensitive fluorescent reporter, HyPer, for cytosol. Cellular H2O2 concentrations peaked at about 5 min and were near baseline by 10 min. The model predicted a skewed distribution of surface areas, with between cell variation usually 2 fold or less. Predicted variability in cellular dose was in rough agreement with the variation in the HyPer data. These results are preliminary, as the model was not calibrated to the morphology of a specific cell type. Future work will involve morphology calibration against human bronchial epithelial (BEAS-2B) cells. Our results show, however, the potential of agent-based modeling to provide a rich description of individual cell dosimetry, with average dose and variability statistics readily derivable. Parallel development of PBPK and virtual tissue models of *in vivo* biology would support a quantitative, biologically-based approach to the alignment of cellular doses *in vitro* and *in vivo*. This abstract does not necessarily reflect any specific policy of the US EPA.

2211 Modeling Concurrent Binding of Mixed Integrin Species to Multiple ECM Ligands

S. V. Hudson, C. E. Dolin, L. G. Poole, V. L. Massey, D. W. Wilkey, G. N. Brock, M. L. Merchant, H. B. Frieboes and G. E. Arteel. *Pharmacology & Toxicology, University of Louisville, Louisville, KY.*

Integrin receptor interactions are key mediators of cell attachment, migration, proliferation, among many other molecular processes, primarily via binding to extracellular matrix (ECM) glycoproteins, leading to bidirectional signal transduction that influences critical cellular functions. The composition of the ECM and the expression of their cognate receptors dictates cell behavior and dynamics. Vital to the maintenance of homeostatic conditions, integrins have become important therapeutic targets for diseases of dysregulation–namely various cancers, immune dysfunction, and chronic inflammation. Promiscuity among the repertoire of ECM ligands and integrin receptors, particularly those with Arg-Gly-Asp (RGD) binding motifs, means binding affinities vary for each combination. Understanding the kinetics of integrin binding can provide a profile of integrin interaction relative to the ECM proteomic signature. Previous work established a mathematical model for deterministic ECM-integrin binding kinetics that incorporates the divalence of the receptor and a simple aggregation scheme to represent clustering. Model parameters related to ligand abundance were based on liver ECM proteomic data and receptor density was taken from published literature. The purpose of the current study is to develop a stochastic model for integrin binding that considers independent and competitive binding of multiple ligands among varied integrin receptor species to recapitulate complex interactions in the intact organism. This model creates a binding profile for known ECM components that will help distinguish patterns between homeostatic and diseased livers, and is predictive of the predominant phenotype of integrin signaling. Applications for this model include characterizing kinetics and dynamics for ECM changes that may influence integrin phenotype.

2212 Oxidative Stress Response - Predicting Adaptive to Adverse Transition

S. Das[3], R. Kumar[3], A. Khanna[3], M. K. Narasimha[3], B. Thota[3], K. Rajendran[3], D. Raman[3], V. Rajagopalan[3], N. Dharwar[3], G. V. Balamurali[3], G. Jain[1], S. Cooper[2], A. Middleton[2], S. Glavin[2], A. White[2], P. Carmichael[2] and K. Subramanian[3]. *[1]SEAC, Unilever, Bangalore, India; [2]SEAC, Unilever, UK, United Kingdom and [3]Life Sciences, Strand Life Sciences Pvt. Ltd, Bangalore, India.*

Oxidative stress can lead to adverse responses including necrosis if a system is unable to adapt to its impact. Being able to quantitatively predict the response to oxidative stress will aid in the understanding of a chemical's liability by improving our capability to define a tipping point between adaptive and adverse doses, or aid in the design of novel antioxidants. We have developed a dynamic systems model of the biological processes involved in ROS generation & quenching, lipid peroxidation & protein oxidation, etc. Perturbing these pathways beyond the ability of NRF2- NFkB signaling to restore homeostasis culminates in oxidative stress induced cell death. We have combined the model with an *in vitro* assay set of enzymes predicted to be key in oxidative stress response including γ-GCS, GR, GPx, JNK, complexes I & II. All the enzyme activities were measured at different doses for a set of 20 compounds. A sub group was used for multi-parameter model optimization by monitoring various biomarkers such as MMP, ROS, nuclear NRF2 & NFkB, cellular MDA & 4HNE representing the progression of oxidative stress, and minimizing a cost function made of multiple experimentally measured values. The model was tested for its ability to predict the dose-dependent effect of curcumin that acts as antioxidant in some regimes and a pro-oxidant in others. We predict that low dose curcumin-primed cells can resist H2O2 and TBH induced oxidative stress compared to unprimed cells - a phenomena validated *in vitro* in HepG2 cells. We predict that at higher concentrations, curcumin induced cell-death originates from altered mitochondrial function. The ability to predict complex effects and system adaptations shows the value of an integrating a validated systems model along with the right biochemical assays - an approach that can predict the exposures likely to tip a system over from an adaptive regime causing adverse effects.

2213 Adverse Outcome Pathway (AOP) Network Development for Fatty Liver

M. M. Angrish[2], J. P. Kaiser[1], C. A. McQueen[2] and B. N. Chorley[2]. *[1]US EPA, Cincinnati, OH and [2]US EPA, Durham, NC.*

Adverse outcome pathways (AOPs) are descriptive biological sequences that start from a molecular initiating event (MIE) and end with an adverse health outcome. AOPs provide biological context for high throughput chemical testing and further prioritize environmental health risk research. According to the Organization for Economic Co-operation and Development guidelines, AOPs are pathways with one MIE anchored to an adverse outcome (AO) by key events (KEs) and key event relationships (KERs). However, this approach does not always capture the cumulative impacts of multiple MIEs on the AO. For example, hepatic lipid flux due to chemical-induced toxicity initiates from multiple ligand-activated receptors and signaling pathways that cascade across biology to converge upon a common fatty liver (FL, also known as steatosis) outcome. To capture this complexity, a top-down strategy was used to develop a FL AOP network (AOPnet). Literature was queried based on the terms steatosis, fatty liver, cirrhosis, and hepatocellular carcinoma. Search results were analyzed for physiological and pathophysiological organ level, cellular and molecular processes, as well as pathway intermediates, to identify potential KEs and MIEs that are key for hepatic lipid metabolism, maintenance, and dysregulation. The analysis identified four apical KE nodes (hepatic fatty acid uptake, *de novo* fatty acid and lipid synthesis, fatty acid oxidation, and lipid efflux) juxtaposed to the FL AO. The apical KEs were further linked through lower levels of biological organization to potential MIEs. A putative fatty liver AOPnet was constructed from MIE, KE, and AO nodes connected by KER edges that contained supporting evidence to infer that an upstream event caused a downstream event. This integrative AOPnet provided an evidence-based framework used to identify a minimal number of *in vitro*-based assays for KEs to infer chemical-induced effects predictive of a fatty liver outcome. From a public health standpoint, limiting chemical exposures that contribute to the etiology of fatty liver is important to prevent serious and potentially progressive liver disease, as well as extra-hepatic effects related to metabolic syndrome and systemic metabolism. This work does not necessarily reflect the policies of the US EPA.

2214 Computational Modeling to Evaluate Alternative Hypotheses for the Linkage of Aromatase Inhibition to Vitellogenin Levels in Fathead Minnows

W. Cheng[3], Q. Zhang[4], A. Schroeder2[1], D. Villeneuve[1], G. Ankley[1] and R. Conolly[2]. *[1]US EPA, Duluth, MN; [2]US EPA, Research Triangle Park, NC; [3]ORISE, Research Triangle Park, NC and [4]The Hamner Institutes, Research Triangle Park, NC.*

Aromatase converts testosterone to estradiol (E2). In fish, E2 concentrations control hepatic synthesis of the glycolipoprotein vitellogenin (VTG), an egg yolk precursor protein essential to oocyte development and larval survival. Fathead minnows were exposed to the aromatase inhibitor, fadrozole, for 8 days and held in control water for an additional 20 days. We observed dose-dependent reductions in plasma E2 and VTG during exposure to fadrozole. While VTG concentrations dropped as the E2 level declined at the onset of exposure, the recovery of VTG during depuration was delayed relative to that of E2. An existing computational model of the hypothalamic-pituitary-gonadal axis was modified to evaluate three alternative hypotheses regarding the regulation of VTG. The first hypothesis, describing the rate of VTG synthesis as proportional to hepatic E2, failed to describe the VTG data. In the second hypothesis, using a type I coherent feed-forward motif, hepatic synthesis of VTG involves both direct E2 signaling and an intermediate transcription factor induced by E2. The third hypothesis involves a negative feedback loop operating in the ovary where the synthesis of the ovarian VTG transporter used for uptake of VTG from the blood is negatively regulated by VTG in the ovary. For both the feedforward and feedback loops, ultrasensitivity, allowing signal amplification and threshold, was implemented using Hill equations. Both the feed-forward and feedback motifs were able to recapitulate the observed VTG dynamics, suggesting that one or both may operate in fathead minnows. While computational modeling cannot reveal the actual biological identity of the regulatory circuits, it can identify hypotheses worth of laboratory investigation. This abstract does not necessarily reflect the policy of the US EPA.

2215 Multiscale Modeling of Aerosol Deposition in the Complete Respiratory Tracts of Animals and Humans

A. P. Kuprat[2], S. Kabilan[2], O. Price[1], B. Asgharian[1] and R. A. Corley[2]. *[1]Applied Research Associates, Raleigh, NC and [2]Pacific Northwest National Laboratory, Richland, WA.*

Computational fluid dynamics (CFD) airflow and material transport modeling is a key approach to predicting site-specific deposition and tissue dose of inhaled materials in the respiratory systems of animals and humans. However, due to limited resolution of MR and CT imaging techniques, especially in humans, 3D CFD models currently extend only into the conducting airways of the lung. Hence CFD-based simulations of aerosols that exit the 3D domain during the inhalation phase of the breathing cycle are assumed to have deposited in the deep lung with no information on location. Furthermore, aerosols that are still in airway lumens of the deep lung are not available for re-introduction to the 3D conducting airways during the exhalation phase. This can lead to over-predictions of deep lung deposition for aerosols and under-predictions of upper respiratory tract deposition and aerosol exhalation, especially those less than a few microns in diameter. In this study, we upgraded our implementation of CFD aerosol modeling using OpenFOAM (a parallel open source 3D CFD software package with Eulerian particle tracking capability) to bi-directionally exchange information with the widely used 1D Multiple Path Particle Dosimetry (MPPD) code that represents particles with a density field. The two-way air and particle transport between 3D and 1D domains is computationally efficient and conserves particle mass and fluid flow. Deposition in both domains is determined by diffusion, sedimentation, and impaction. The resulting model provides site-specific deposition in the 3D domain and regional airway deposition in the 1D domain in the complete respiratory system of rats and humans. Details of our coupling of OpenFOAM and MPPD codes will be shown along with comparisons with published experimental results. This approach is extensible to other species where imaging-based 3D CFD models are available. Furthermore, the human models can be individualized with 3D imaging to facilitate personalized therapeutic approaches. Funded by NHLBI R01 HL073598.

2216 Comparison of Realistic and Idealized Breathing Patterns in Computational Models of Airflow and Vapor Dosimetry in the Rodent Upper Respiratory Tract

S. M. Colby, S. Kabilan, R. E. Jacob, A. P. Kuprat, D. R. Einstein and R. A. Corley. *Pacific Northwest National Laboratory, Richland, WA.*

Computational fluid dynamics (CFD) simulations of airflows coupled with physiologically based pharmacokinetic (PBPK) modeling of respiratory tissue doses of airborne materials have traditionally used either steady-state inhalation or a sinusoidal approximation of the full breathing cycle as the driver for airflow simulations despite their differences from normal breathing patterns. We therefore evaluated the impact of multiple breathing profiles on tissue dosimetry with a published CFD/PBPK model for rats exposed to acetaldehyde at the NOAEL (50 ppm) for nasal toxicity. Simulations using measured normal respiration and sniffing profiles derived from whole-body plethysmography studies with male Sprague-Dawley rats were benchmarked against an idealized, sinusoidal profile at comparable minute volumes to evaluate differences in site-specific time- and tissue depth-dependent areas under the curve (AUC) for acetaldehyde in each region of the nose. For the normal ventilation pattern, a reduction of 10 to 40% in local AUCs were predicted in wet squamous, respiratory and transitional epithelium along the main airflow path with a corresponding increase in AUCs in olfactory epithelium, especially the most distal regions of the ethmoid turbinates vs. the idealized profile. However, the differences in AUCs at known lesion forming regions (anterior respiratory/transitional epithelium and anterior dorsal olfactory epithelium) between normal and idealized profiles were minimal, suggesting that sinusoidal profiles may be used for this chemical and exposure concentration. As expected, the higher amplitude/higher frequency sniffing profile produced greater increases (100-500% over idealized profile) in local AUCs in the olfactory epithelium, especially in the posterior region. Thus, depending upon the chemical, exposure system, and the time spent sniffing, this behavior could become an important contributor to tissue dose predictions. Funded by NHLBI Grant no. R01 HL 073598.

2217 Development of a Mathematical Model of Anthrax in Humans and Application to Estimate Risk to Fielded Biological Threat Identification Equipment Operators

C. E. Hack[1], J. M. Gearhart[1], T. R. Covington[1], C. R. Starr[2] and R. R. Chapleau[2]. [1]*711 HPW/RHDJ, Wright-Patterson AFB, OH and* [2]*Aeromedical Research, USAFSAM, Wright-Patterson AFB, OH.*

Current DoD protocols for isolating genetic material from field samples to identify biological threats include a step termed "bead-beating" to destroy cell walls or spore coat to release DNA for easier identification. However, it is unknown how many intact viable spores remain following this procedure, and what risk is posed to field personnel. Two alternative chemical treatments have been evaluated in an effort to improve this process. We modified an existing mathematical model of rabbit anthrax infection to assess the comparative risk of infection in humans associated with the standard methodology and the two chemical supplements identified as potential replacements. Measurements were made of viable *Bacillus anthracis* (Ba) spore concentrations released into the air during processing of samples following "bead-beating" versus each of the chemical treatments. The physiological parameters of the mathematical model were modified to be representative of humans, enabling the simulation of the human response. The modified model includes spores, vegetative bacteria, and macrophages in the lung airways, alveolar surface, lymph system, and blood. Predictions of alveolar deposition of spores, bacteremia, and lung and lymph bacteria were calculated and compared across the different treatments. The model was exercised to predict the effects of various workloads on lung deposition and bacterial proliferation. The estimates were compared with the anthrax dose-response literature (e.g., Haas), and compared to the biological military exposure guideline (BMEG) for anthrax. The results of this research will provide scientific basis for field operation decisions, and reduce the risk to field operators.

2218 Systems Toxicology Modeling for a Compound That Caused Drug Induced Liver Injury (DILI) in Humans but Not Preclinical Species

H. A. Barton[3], M. D. Aleo[3], B. Howell[1], Y. Yang[1], S. Siler[1], P. B. Watkins[1] and L. Shoda[1]. [1]*Hamner Institute for Health Sciences, Research Triangle Park, NC;* [2]*Pfizer, Inc., Groton, CT and* [3]*DSRD, Pfizer, Inc., Groton, CT.*

Drug candidates that cause DILI in clinical trials with little or no evidence of liver injury in preclinical species are problematic in pharmaceutical development. One such example is PF-04895162 (ICA-105665), a Kv7 ion channel modulator. Preclinical studies in rats and cynomolgus monkeys showed essentially no liver toxicity, whereas elevations in alanine aminotransferase (ALT) were observed in a small 2 week multi-dose clinical study. Systems toxicology simulations were conducted using the DILIsym® software to assess interspecies differences between rats and humans. Data from rat and human pharmacokinetic (PK) studies were used to parameterize a physiologically based PK sub-model to estimate liver concentrations. *In vitro* data characterizing inhibition of human bile salt export pump (BSEP) and decreases in mitochondrial electron transport chain activity in human hepatocytes provided the basis for assessing these potential mechanisms of toxicity. Simulation results recapitulated species differences in hepatotoxicity resulting from mitochondrial impairment and alterations in bile acid homeostasis. For humans, it predicted greater severity of effect but lower incidence than observed in the study (2-200x vs. 2-10x ALT; n=23/212 vs. 6/8). Mitochondrial dysfunction was an important contributor to predicted toxicity, though it was essential to use measured compound accumulation in hepatocytes rather than media concentration. While the type of BSEP inhibition was not determined experimentally, non-competitive inhibition led to 23/212 human ALT elevations; whereas competitive inhibition reduced this effect to 2/212. These analyses help identify appropriate *in vitro* data to maximize the predictivity of the model. For compounds altering mitochondrial function and bile acid homeostasis, combining *in vitro* data and a computational model provides a powerful approach to evaluate potential human DILI even in the absence of preclinical evidence.

2219 Development of Physiologically Based Pharmacokinetic (PBPK) Models for the Quantitative Characterization of Interspecies Oseltamivir Pharmacokinetics

N. Fausto[1,2], L. Loukotkova[2], G. Gamboa[2] and A. Lumen[2]. [1]*College of Pharmacy and Health Sciences, St. John's University, Queens, NY and* [2]*Division of Biochemical Toxicology, NCTR/US FDA, Jefferson, AR.*

Oseltamivir is an oral anti-viral drug approved for the treatment and prevention of influenza. Numerous physiological changes occur in pregnancy that may result in the under-dosing of oseltamivir in pregnant women. The overall goal of this study is to evaluate the utility of animal models in conjugation with PBPK models to predict the pharmacokinetics of oseltamivir in pregnant women. The current work focuses on characterizing the interspecies differences and similarities of oseltamivir pharmacokinetics in non-pregnant humans and rhesus monkeys. Preliminary pharmacokinetic experiments were conducted in rhesus monkeys dosed with oseltamivir under non-pregnancy conditions. First-generation PBPK models for the non-pregnant humans and rhesus monkeys were developed. The models include PBPK sub-models for oseltamivir and its active carboxylate metabolite. Parameter sensitivity analyses were conducted in order to verify model structure and identify key parameters for model optimization. Refinement of the human model was implemented based on pharmacokinetic data acquired from the literature for multiple doses and routes of exposure. The human PBPK model predictions of the active metabolite's pharmacokinetic profile were in good agreement with the observed data used exclusively for model evaluation. Similarly, a non-pregnant animal PBPK model was developed and calibrated using in-house experimental pharmacokinetic data. Our preliminary findings using the models suggest that the utility of an animal model in predicting an endpoint of interest for oseltamivir in humans is driven by the mechanism that governs the rate-limiting step amidst the overall pharmacological differences between the species. Parameters determining the rate-limiting step were identified and a cross-species extrapolation is currently being developed. Knowledge gathered from these PBPK models will be used to develop a comprehensive PBPK model network that has the ability to extrapolate across species and life-stages to predict safe and effective dosing of oseltamivir during pregnancy in humans.

2220 Pharmacovigilance Activities in Children

E. Ozcagli and B. Alpertunga. *Istanbul University Faculty of Pharmacy, Istanbul, Turkey.*

Pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects, or any other drug-related problem. In recent years pharmacovigilance activities mostly focused on risk management strategies, vulnerable patients, orphan drugs, individual medications and novel technologies. Due to ethical and protective attitude, children often excluded from clinical trials unless the drug has indications specifically on children. We often have limited access regarding information on efficacy, risks and dose recommendations specifically determined for age group. Limitations provoke off-label usage and it is known that off-label usage in paediatric population probably increases the risk of adverse drug reactions. Children differ vastly within their own age group and prescribing age-suitable doses is a new obstacle for appropriate paediatric treatments. In this study, we have conducted a search of papers published until October 2015 by crossing keywords and investigated several biological and physical issues and important risk factors which may contribute drug safety in children. As a result, developmental factors and drug therapy complications are defined as major parameters and effective pharmacovigilance activities proposed as a solution for paediatric drug safety.

2221 Evaluating the Value of Augmenting *In Vitro* Hazard Assessment with Exposure and Pharmacokinetics Considerations for Chemical Prioritization

C. Tan[2], J. Leonard[2], S. Scholle[2], M. Winter[2], P. Key[2], J. Lu[2] and D. Chang[1]. [1]*Chemical Computing Group, Montreal, QC, Canada and* [2]*US EPA, Research Triangle Park, NC.*

Over time, toxicity-testing paradigms have progressed from low-throughput *in vivo* animal studies for limited numbers of chemicals to high-throughput (HT) *in vitro* screening assays for thousands of chemicals. Such HT *in vitro* methods, along with HT *in silico* predictions of population exposure levels and pharmacokinetic (PK) properties, act as the foundation for HT risk assessment. The impact of uncertainties in-

herent in predicted exposure levels and PK behaviors has not been evaluated in overall risk estimates, however. In the current study, this impact was investigated using a medium-throughput approach that integrates exposure, PK, and hazard data using a one compartment PK/pharmacodynamic (PD) model to prioritize 25 acetylcholinesterase (AChE) inhibiting chemicals. The PK model was used to generate chemical steady state plasma concentrations (Css), which were then used together with *in vitro* potency data in the PD model to predict inhibition activities and place these chemicals into five priority classes. Compared to a reference scenario in which all model parameters were based on measured values, the scenario in which clearance values were predicted using *in silico* models resulted in 29% misclassification. In contrast, scenarios in which measured clearance values were used in conjunction with absorbed doses predicted using a HT exposure model resulted in 70% misclassification, and scenarios using predicted values for both clearance and exposure resulted in 74% misclassification. Our findings suggest that appropriate estimates of exposure levels generate a higher degree of confidence in chemical prioritization. [Disclaimer: This abstract has been cleared by the EPA but solely expresses the view of the authors.]

2222 Integrating High Throughput Exposure and Hazard Data Using a Physiologically Based Pharmacokinetic/pharmacodynamic Model for Thyroid Peroxidase Inhibitors

J. Leonard, C. Tan, M. Gilbert, K. Isaacs and H. El-Masri. *US EPA, Research Triangle Park, NC.*

High throughput (HT) *in vitro* toxicity testing, along with HT *in silico* exposure predictions, hold the promise of conducting human health risk assessment with greater speed and require less resources. The lack of a complete biological system in these HT approaches, however, precludes the incorporation of pharmacokinetics. In this study, a physiologically based pharmacokinetic (PBPK) model was developed and linked to a pharmacodynamic (PD) model to integrate *in vitro* potency data, *in silico* exposure predictions, and pharmacokinetic characteristics for estimating margin of exposure (MOE) for six thyroid peroxidase (TPO) inhibitors. These chemicals ranged in potency from high (methimazole and 6-propylthiouracil) to moderate (benzophenone-2 and 2-mercaptobenzothiazole) to low (resorcinol and triclosan). The PBPK/PD model described TPO inhibition as a function of binding affinity, which was determined through *in vitro* testing, and chemical concentration within the thyroid gland. The model was then used to estimate human equivalent intake doses able to inhibit thyroid hormone levels by 10% (ED10) for each chemical. Next, MOE was calculated by comparing each ED10 value to population exposure levels predicted from a HT exposure model. Results of this case example revealed that chemical potency and MOE estimates might not necessarily be related. For example, benzophenone-2 and triclosan exhibited small MOE values due to slow clearance or high exposure potential, respectively, for each chemical. Our study suggests that prioritizing chemicals based on hazard or exposure alone may not be sufficient, and that integration of exposure, hazard, and pharmacokinetics is warranted. Disclaimer: This abstract has been cleared by the US EPA but solely expresses the view of the authors.

2223 *In Vitro-In Vivo* Extrapolation (IVIVE) to Support *In Silico/In Vitro* Based Risk Assessment: PBTK Modeling of Selected Potential Endocrine Modulators

C. Haase, E. Fabian, T. Ramirez, B. van Ravenzwayy and R. Landsiedel. *Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

Physiologically Based Toxicokinetic Modeling (PBTK) is an *in silico* tool to predict compound kinetics based on physiological parameters of the organism and test substance related properties. PBTK is a key element for inverse dosimetry to relate effect concentrations *in vitro* to external, e.g. oral doses. In our investigations, we use 8 compartment models for the rat including adrenals and testes or ovaries. Test substance specific properties taken for PBTK modeling are molecular weight and logPO/W as well as IVIS based tissue specific partition coefficients, hepatic clearance, intestinal permeability and plasma protein binding. Berkeley Madonna Software was used to solve the consequent differential equations. We evaluated the above described model for the 3 test substances bisphenol A (BPA), fenarimol (FEN) and ketoconazole (KETO). Using the lowest effect concentrations (LOECs) of BPA, FEN and KETO from 1) an *in vitro* yeast based assays with human estrogen and androgen receptor combined with a reporter gene and 2) the interaction of steroidogenesis model calculations were made to relate *in vitro* concentrations to oral doses in the rat. Model calculations, based on *in vitro* LOECs of 10 µM (BPA), 3 µM (FEN) and 0.01 µM (KETO), for concentrations in target organs resulted in estimated oral LOELs of 16, 4 and 0.04 mg/kg. When calculations were made for plasma levels oral LOELs were estimated to be 608, 77 and 16 mg/kg for BPA, FEN and KETO, respectively When compared to existing *in vivo* data with endocrine related LOELs of 375 mg/kg bw day for BPA (1), 50 mg/kg day for FEN (2) and 6 mg/kg day for KETO (3), it can be concluded that IVIS related risk assessment approaches based on target tissues seems overpredictive, whereas plasma related LOELs were closer to the *in vivo* situation. 1 Kanno J. et al. (2003) Environ Health Perspect 111, 785-794. 2 US EPA (2007) http://www.epa.gov (26.09.2015). 3 Shin J-H. et al. (2006) Arch Toxicol 80, 797-803.

2224 *In Vitro* to *In Vivo* Extrapolation for Estrogenic Activity of Environmental Chemicals

X. Chang[3], N. Kleinstreuer[3], P. Ceger[3], N. Choksi[3], J.-H. Hsieh[1], B. A. Wetmore[4], S. Ferguson[2], M. DeVito[2], D. Allen[3] and W. Casey[3]. *[1]Kelly Services, Inc., Durham, NC; [2]NIEHS/DNTP, Durham, NC; [3]NIEHS/NICEATM, Durham, NC and [4]The Hamner Institutes for Health Sciences, Durham, NC; [5]ILS, Inc., NIEHS/NICEATM, Durham, NC.*

In vitro high-throughput screening (HTS) assays provide an efficient way to identify potential estrogen-active chemicals. To best utilize *in vitro* HTS assay data in prioritization and regulatory decision making, *in vitro* to *in vivo* extrapolation (IVIVE) is necessary. In this study, we evaluated the impact of critical pharmacokinetic parameters, dosimetry, and modeling approaches on IVIVE of estrogenic activity. *In vitro* lowest effective concentrations (LECs) for ten chemicals positive for estrogen receptor (ER) agonist activity were obtained from each of 16 ToxCast and/or Tox21 HTS assays mapping to various key events along the ER pathway. One-compartment and multi-compartment physiologically based pharmacokinetic models were used to estimate daily equivalent administered dose (EAD) that would result in blood concentrations equivalent to the *in vitro* LECs. The estimated lowest or median EADs were compared to the lowest or median lowest effect level (LEL) from *in vivo* uterotrophic assays for each chemical. The influence of fraction of unbound chemical (Fub) and hepatic clearance on EAD estimates was evaluated by systematically varying these parameters across a range of observed values. In addition, we applied a Fub adjustment method to better estimate the free fraction of a chemical available for tissue/cell uptake. We observed close agreement between EADs and *in vivo* LELs for the majority of chemicals tested, particularly after Fub adjustment. This study demonstrates an optimized approach for using *in vitro* data to quantitatively predict *in vivo* effects. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

2225 Evaluation of Pharmacokinetic Assumptions Using a 443 Chemical Library

R. Pearce[2], W. Setzer[2], J. Davis[1,2] and J. Wambaugh[2]. *[1]Syngenta, Research Triangle Park, NC and [2]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC.*

With the increasing availability of high-throughput and *in vitro* data for untested chemicals, there is a need for pharmacokinetic (PK) models for *in vitro* to *in vivo* extrapolation (IVIVE). Though some PBPK models have been created for individual compounds using *in vivo* data, we are now able to rapidly parameterize generic PBPK models using *in vitro* data to allow IVIVE for chemicals tested for bioactivity via high-throughput screening. However, these new models are expected to have limited accuracy due to their simplicity and generalization of assumptions. We evaluated the assumptions and performance of a generic PBPK model (R package "httk") parameterized by a library of *in vitro* PK data for 443 chemicals. We evaluate and calibrate Schmitt's method by comparing the predicted volume of distribution (Vd) and tissue partition coefficients (PCs) to *in vivo* measurements. The PCs are initially over predicted, likely due to overestimation of partitioning into phospholipids in tissues and the lack of lipid partitioning in the *in vitro* measurements of the fraction unbound in plasma. Correcting for phospholipids and plasma binding improved the predictive ability (R2 to 0.52 for PCs and 0.32 for Vd). We lacked enough data to evaluate the accuracy of changing the model structure to include tissue blood volumes and/or separate compartments for richly/poorly perfused tissues, so we evaluated the impact of these changes on model outputs. We examined the duration (tdist) and concentration at the end of the distribution phase, elimination rate, AUC, maximum concentrations, number of days to steady state, and time elapsed for 90% of chemical eliminated. We found that the only significant change in model outputs is in tdist. The richly/poorly perfused correction doubled tdist while for the blood volume correction tdist was ¾ of the original. These model changes also increased the predicted value for Vd at least two times for 153 chemicals. Overall, comparison to *in vivo* data identified discrepancies that we reduced by refining our phospholipid partitioning and plasma binding models, which improved

the accuracy of the PC predictions. However, separation of the rest of body into richly/poorly perfused compartments and consideration of blood volumes only made a significant difference in the duration of the distribution phase. This abstract does not necessarily reflect US EPA policy.

2226 Multi-Route Toxicokinetic Predictions with GastroPlus™ Software for High-Throughput Exposure Assessments

L. Luna[2], N. Sunger[1], B. Bhhatarai[2], F. Zhang[2], D. Wilson[2], S. Arnold[2], A. Beasley[2], B. Landenberger[2] and M. Bartels[2]. *[1]West Chester University, Philadelphia, PA and [2]Toxicology, Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.*

Background: New high-throughput exposure and risk evaluation programs rely on *in silico* tools for the prediction of biological responses to a variety of chemicals, including the prediction of absorption and/or bioavailability. Alternatives to animal experiments such as predictive software tools are therefore being developed, and regulatory frameworks are providing an increasing responsibility to use such methods. In this study, the Quantitative Structure-Activity Relationship (QSAR) software GastroPlus™ was utilized to determine bioaccumulation factors (BAF) as well as fraction absorbed by oral, inhalation and dermal routes of exposure in humans. These values will then be incorporated into a High-throughput Exposure Assessment Tool (HEAT). Method: BAF estimates for 60 chemicals representing various physical/chemical characteristics were generated with a PBPK model, to accommodate nonlinearities in absorption and CYP-based metabolism, for repeat exposures via oral, inhalation or dermal routes. Blood Cmax values were then simulated from acute exposures by all three routes, at levels spanning low, environmentally-relevant levels to those used in high-dose toxicity studies. Results: BAF values correlated fairly well with lipophilicity of chemicals, for all three exposure routes. Systemic exposure predictions were generally linear with dose, with some exceptions for compounds that have saturable absorption. Accuracy of the predictions was acceptable (within 3x of empirical for 20-compound subset), especially for poorly metabolized compounds that are not substrates for transporter systems. Conclusion: Multi-route toxicokinetic predictions with GastroPlus provide a means of extending high-throughput exposure tools to systemic levels. These data will then be very useful for screening-level assessments of chemical product safety, based on *in vitro* toxicity assay programs or *in vivo* animal testing with integrated toxicokinetic endpoints.

2227 Applications of the PBPK Model of Dioxins to Evaluate Real Life Exposure Scenarios

C. Emond[2], P. Ruiz[3] and M. Mumtaz[3]. *[1]ATSDR/CDC, Atlanta, GA; [2]BioSimulation Consulting Inc., Newark, DE and [3]DTHHS, ATSDR/CDC, Atlanta, GA.*

Previously, we have presented human physiologically based pharmacokinetic (PBPK) models recoded in Berkeley Madonna (BM) software for several priority pollutants. This translational research project envisioned development of a series of PK/PBPK models as a tool for risk assessors. Presented here is a recoded human model used by the U.S. EPA for their recent Dioxin assessment. The model was applied to two documented real life dioxins exposures in populations (1) living in Calcasieu (potentially contaminated) and Lafayette (non-contaminated) parishes in Louisiana (USA) and (2) near a hazardous waste incinerator in Tarragona County, Catalonia, Spain. In the first case, serum dioxins levels following chronic lifetime exposure simulations as a function of age, showed a linear association (R2=0.9252) suggesting age as a determining factor. The oldest age group, >60 years, had the highest levels suggesting accumulated exposures over the years. However, compared to the Lafayette population, the Calcasieu Parish population did not significantly higher exposed. In the second case, 15 individuals from the Spanish cohort showed a decreasing trend in dioxin equivalents levels suggesting a significant reduction in dioxin intake. The highest and lowest levels found were 46.3 pg WHO-TEQ/g fat (72 year old individual) and 5.1 pg WHO-TEQ/g fat (30 year old individual), respectively. These observations are in agreement with the observations made in 1998 (32.1±15.3) and 2013 (11.5±11.1) showing a WHO-TEQ decrease of 65%. Ongoing biomonitoring and PBPK modeling efforts support these findings. Experience gained through such exercises creates the potential for generation of reverse-exposure calculations using serum dose metrics as a surrogate.

2228 Simulated Reconstructions of High Performance Aircraft Pilot Exposures Using Physiologically Based Pharmacokinetic Modeling

H. A. Pangburn[3], T. Covington[2], T. Habib[3], R. S. Mayes[4], C. C. Grigsby[1], D. K. Ott[4] and J. M. Gearhart[2]. *[1]711th Human Performance Wing/RHXBC, WPAFB, OH; [2]Henry M. Jackson Foundation, WPAFB, OH; [3]STS on contract to US Air Force School of Aerospace Medicine (USAFSAM), WPAFB, OH and [4]USAFSAM, WPAFB, OH.*

Chemical irritants can pass through the on-board oxygen generation system in high-performance aircraft (HPA), but the level and further identification of irritants have not been thoroughly evaluated. The 711th Human Performance Wing recently conducted a High-Performance Aircraft Respiratory Study (HPARS) to identify the etiology of reported respiratory pilot symptoms and mitigate a common health complaint in HPA. HPARS sampling included exhaled breath before and after hundreds of sorties to identify volatile organic compunds (VOCs). Typically, VOCs absorbed into the body via inhalation rapidly clear from the bloodstream after cessation of exposure. Thus, it is challenging to accurately reconstruct actual exposure concentrations and blood levels within a pilot's circulatory system and potential toxic chemical levels at the most responsive tissue sites (i.e., lung and central nervous system). The current study seeks to overcome these issues by applying physiologically based pharmacokinetic modeling to the HPARS exhaled breath samples to reconstruct pilot blood and tissue concentrations during each specific sortie. These reconstructions allowed us to determine possible exposure ranges experienced by pilots during flight (some modeled in-flight concentrations exceeded post-sortie breath samples as much as 1000x), compare modeled exposure to permissible exposure limits and threshold limit values (TLVs) (some modeled in-flight concentrations exceeded TLVs), and compare modeled exposure to exposure levels and symptomology from the literature associated with various chemical species. This approach produced informed knowledge judgment as to the probability that any particular chemical could have both the potential to have achieved high enough in-flight exposure concentrations and have a known level of toxic potency to be of consequence.

2229 Reverse Dosimetry Modeling of Toluene Exposure Concentrations Based on Biomonitoring Levels From the Canadian Health Measures Survey

H. G. Tohon[3], M. Moreau[1], M. Valcke[2], S. Haddad[3] and A. Nong[1]. *[1]Occupational and Environmental Health, Health Canada, Ottawa, ON, Canada; [2]Occupational and Environmental Health, National Institute of Public Health of Quebec, Montreal, QC, Canada and [3]Occupational and Environmental Health, University of Montreal, Montreal, QC, Canada.*

Biomonitoring is a popular measure of environmental chemicals for a group of individuals that can represent the exposure of a larger population. Nationwide surveys such as the National Health and Nutrition Examination Survey and the Canadian Health Measures Survey can provide useful estimates of population exposure. However, data uncertainties such as interindividual variability are common in large population surveys. Physiologically-based pharmacokinetic (PBPK) models can be used to simulate population by incorporating different variability factors. The objective of this work is to reconstruct residential indoor air concentrations of toluene from blood measures reported in the Canadian Health Measures Survey using reverse dosimetry PBPK modeling techniques. Population distributions of physiological parameters are described based on age, weight and size. Monte Carlo simulations with the PBPK model were used to generate Exposure Conversion Factor tables to convert distribution of blood measures into indoor air levels. Based on blood levels observed at the 50th, 90th and 95th percentiles, toluene residential indoor air concentrations of toluene were estimated as respectively: 0.013, 0.035 and 0.063 ppm in adults aged 20-39 years, 0.021, 0.049 and 0.091 ppm for those aged 40-59 years, and 0.016, 0.044 and 0.082 for those aged 60–79 years. These estimated distributions of toluene concentrations in residential indoor air are well below residential indoor air guidance levels (i.e., 0.6 ppm for neurological effects). PBPK modeling and reverse dosimetry can be combined to help interpret biomonitoring data and to prioritize management efforts for the exposure assessment of environmental chemicals.

2230 How Malathion Dermal Absorption Varies with Dermal Load, Based on Physiologically Based Pharmacokinetic Modeling of *In Vivo* Human Data

A. Singhal. *Exponent, Inc., Suite 400, CA.* Sponsor: K. Bogen.

Absence of a validated method to scale estimated rates of chemical-specific dermal uptake to apply to widely differing conditions of applied dermal load L (mg/cm2) can impede realistic dermal exposure assessment. Using *in vitro* methods and using a (perhaps physically unrealistic) model of observed apparent 1st-order rate (rp) of dermal absorption (rp = a[L cm2/mg]-b hour-1), estimates of -0.374, -0.478, and -0.463 were reported for values of slope b specific to the organophosphate pesticides (OPs) fenthion, methyl parathion, and parathion, respectively. Comparable relationships estimated from *in vivo* data are not available for any chemical. Here we report the first similar estimate based on a re-analysis of 29 sets of data reported in previous *in vivo* human studies of human dermal uptake of malathion and corresponding urinary metabolite excretion. Using an updated human physiologically based pharmacokinetic (PBPK) model for malathion to compare rates rp estimated from those data to corresponding experimental values of L, we estimated a highly predictive corresponding (log-log) regression relationship, rp = 0.00341 (L cm2/mg)-0.352 hour-1 (p = 10-6), which includes a log-log slope estimate (b = -0.352) similar to those of three OPs obtained by *in vitro* methods. However, we also found the malathion data to be consistent with an alternative and likely physically more realistic interpretation that malathion has a dermal permeability coefficient, Kp, eff (cm/hour) that only apparently decreases over time associated with a reduction in contacted dermal surface area over time at a rate inversely proportional to L. A provisional approach to extrapolating the rp-vs.-L relationship estimated for malathion to other dermally contacted pesticides is proposed, requiring only a single {rp, L} observation for each new chemical, until new data allow improved predictions.

2231 PBPK-Model Estimates of Bromodichloromethane (BDCM) Delivery to the Human Urinary Bladder

E. M. Kenyon, C. Eklund, J. E. Simmons and R. A. Pegram. *US EPA, Durham, NC.*

Recent data indicate that noningestion exposure to trihalomethanes (THMs), including BDCM is highly correlated with urinary THM levels. Characterizing urinary levels of drinking water disinfection byproducts (DBPs) will likely be important for understanding DBP-associated bladder cancer. Non-oral exposures contribute significantly to the amount of BDCM available for distribution to target tissues (e.g., bladder urothelium). We refined our multi-route human BDCM PBPK model to include urine production in the kidney, urine retention in bladder lumen and delivery to the bladder via both blood and urine. The revised model was able to adequately predict urinary BDCM concentrations from water use studies in the published literature. Predicted internal dose metrics relevant for bladder exposure are area under the curve (AUC) and maximum concentration (Cmax) for BDCM in arterial blood flow to bladder tissue, in bladder tissue and in urine. These metrics were compared for oral and shower related (inhalation + dermal) exposures to water containing 10 ppb BDCM. For all metrics, noningestion exposure (e.g., showering) resulted in 25- to 35-fold higher values compared to ingestion indicating the importance of dermal and inhalation routes of exposure as potential contributors to bladder tissue exposure. For a 10 minute shower, AUC and Cmax were 40-fold and 100-fold higher, respectively for BDCM delivered in urine compared to BDCM delivered by blood to bladder tissue. When a liter of water is ingested over a 10 minute period, AUC and Cmax were 45-fold and 130-fold higher, respectively, for BDCM in urine compared to blood-delivered BDCM. These data suggest the potential for BDCM delivery to bladder in the urine as an important contributor to urothelial exposure and suggests the value of measuring urinary BDCM as a toxicologically relevant measure of dose to target tissue. (This abstract does not reflect Agency policy).

2232 Route-to-Route Extrapolation of 1,2-Dichloroethane Studies from the Oral Route to Inhalation Using Physiologically Based Pharmacokinetic Models

M. L. Gargas and L. M. Sweeney. *Independent Consultants, Dayton, OH.*

To help develop a comprehensive, quantitative understanding of the hazards of 1,2-dichloroethane (ethylene dichloride, EDC, CAS No. 107-06-2) exposure by the inhalation route, the results of existing subchronic studies and an extended one-generation reproductive toxicity (EOGRT) study, recently conducted in rats by the oral route, were extrapolated using a physiologically based pharmacokinetic (PBPK) model. The no observed adverse effects levels (NOAELs) for the endpoints of neurotoxicity and reproductive/developmental toxicity were the highest tested doses of 169 and 155 mg/kg/day, respectively. The NOAELs in rats exposed by drinking water in the neurotoxicity and reproductive/developmental cohorts were equivalent to continuous exposure of rats to minimums of 76 ppm and 62 ppm EDC, respectively, based on PBPK extrapolation using total metabolism of EDC as the dose metric that is equivalent in the oral and inhalation scenarios. In contrast, the subchronic study NOAEL of 37.5 mg/kg/day by gavage corresponded to continuous inhalation exposure of 4.4 ppm EDC, based on equivalent extrahepatic metabolism. The selection of the internal dose metric, which serves to establish route-to-route equivalency, was found to profoundly influence the NOAEL-equivalent inhalation exposure concentration and thus will be a key determinant of inhalation toxicity reference criteria developed on the basis of EDC studies conducted by the oral route. Funded by the EDC HAPs Task Force.

2233 Alcohol Calculations in a Stage 5 Transgender Female

M. D. Dulaney. *Dulaney Toxicology Inc, Tallahassee, FL.*

Introduction: This was a Dram Shop case alcohol case involving death by falling in a fully transformed Stage 5 female. Several competing alcohol consumption patterns were presented by various alcohol-serving establishments. Knowns: Food consumption pattern, visual impressions over time by trained alcohol observers, a BMI calculated at a gym two weeks prior (methodology unknown), and serum alcohol levels at hospital admission of 269 g/dL and 245 g/dL 1.5 hours later. There was also significant blood loss. Physically her body weight had decreased by about 20 pounds during the transformation period. Estrogen replacement therapy was complete and she was using aldosterone for estrogen maintenance. Physical appearance and muscle distribution strongly resembled a young female. Objectives: 1) Develop realistic Widmark rho and beta values for use in BAC calculations using a peer-reviewed blood alcohol model. 2) Evaluate the various drinking patterns over time. 3) Determine the total amount of alcohol served to her before her accident. Results: 1) Historical Widmark rho and beta vales were the least successful. She was neither fully nor fully female. 2) Values based on her gym BMI were also unsuccessful. 3) Values based on calculated BMI were a much better fit. 4) Values based on Total Body Water as an average of male and female values was the most successful. Conclusions: 1) Calculations based on purely male or female Widmark values are likely to be unsuccessful. 2) BMI values are better. 3) Total Body water provided the best fit to the known data. 4) Alcohol calculations transgender individual will likely require a multi-step process in order to accurately describe a Dram Shop situation.

2234 Utilization of Physiologically Based Pharmacokinetic Modeling (PBPK) to Understand the Role of Fetal Metabolism in Polycyclic Aromatic Hydrocarbon (PAH) Toxicity

J. Smith[2], S. R. Crowell[1], D. Mehinagic[2], N. C. Sadler[2], A. T. Wright[2] and R. A. Corley[2]. [1]*Genentech, South San Francisco, CA and* [2]*PNNL, Richland, WA.*

Dibenzo[def,p]chrysene (DBC) is a PAH that causes cancer in offspring of mice exposed during gestation. Like many PAHs, DBC requires metabolic bioactivation to form the ultimate toxicant. Cytochrome P450 enzymes hydroxylate DBC and metabolites (e.g. dibenzo[def,p]chrysene-11,12 diol (DBC diol)) to form epoxide and diol-epoxide intermediates, respectively, which are reactive and can form adducts with DNA. To better understand the internal dosimetry of these compounds and what factors contribute to the risk of DBC toxicity, a combination of experimental and modeling approach was applied to DBC. Pregnant B6129SF1/J mice were exposed to 15 mg/kg DBC by oral gavage, and subgroups of mice were sampled up to 36 hr post dosing. DBC and its diol and tetrol me-

tabolites were quantified in fetal and maternal blood and tissues using high performance liquid chromatography with a fluorescence detector. An existing PBPK for DBC in pregnant mice was expanded to include DBC diol metabolism measured in adult hepatic microsomes, DBC diol metabolism measured in fetal hepatic microsomes, and target tissue dosimetry in fetal tissues. It has been hypothesized that the susceptibility of fetuses maybe partially dependent aryl hydrocarbon receptor (AHR) responsive phenotypes. After 24 hr post DBC exposure, activity-based protein profiling (ABPP) analysis indicated induction in the amount of active P450s in fetuses that were AHR-responsive compared to fetuses that were not, suggesting that overall fetal metabolic capacity are altered in susceptible individuals. Only after including fetal metabolism was the PBPK model able to accurately simulate DBC disposition in the fetus, highlighting the importance fetal metabolism. Overall these results suggest that fetal metabolism of DBC plays an important role determining risk to PAHs, and PBPK models may serve as a platform to extrapolate to humans. Funded by NIEHS Grant No. P42 ES016465.

PS 2235 Evaluating the All-ages Lead Model Using Site-Specific Data: Approaches and Challenges

E. D. McLanahan[1], L. Wilder[1], D. Jackson[1], D. Mellard[1], K. Scruton[1], K. Bradham[2] and R. Worley[1,3]. *[1]CDC/ATSDR, Atlanta, GA; [2]US EPA/ORD/NERL, Research Triangle Park, NC and [3]Univ of Georgia, Athens, GA.*

Lead (Pb) exposure continues to be a problem in the United States. Even after years of progress in reducing environmental levels, CDC estimates at least 500,000 US children ages 1–5 years have blood Pb levels (BLL) above the CDC reference level of 5 ug/dL. Childhood Pb exposure is associated with neurological consequences and public health professionals continue to work to reduce Pb exposures. To better understand the relationship between exposure and BLL, the US EPA has developed a beta version of the All-Ages Lead Model (AALM). Compared to the Integrated Exposure Uptake Biokinetics (IEUBK) Model for lead in children, the AALM provides greater flexibility to describe Pb exposures (acute or chronic, constant or intermittent) for any age. At this time, the AALM has the capability to use three sources of exposure in each of the following media: dust/soil, water, air, food, and other. As part of an interagency test group, we evaluated the ability of the AALM beta v4.2 (Leggett version) to predict BLLs for children that were exposed to Pb in their environment near the John T. Lewis and Bros Lead Smelter Superfund site and met our inclusion criteria. Fourteen children were identified that spent less than 20 hours a week away from the home (e.g., no daycare or school) and had paired BLL and environmental sampling data (i.e., Pb in soil, window sill dust, front door dust, floor dust, and drinking water). The model can predict average BLLs, but it remains difficult to predict individual level BLLs due to a number of uncertainties associated with characterizing lead exposures. The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry or the US Environmental Protection Agency and should not be construed to represent any agency determination or policy.

PS 2236 Comparison of All Ages Lead Model Version 4 with LeadSpread 8 in Evaluation of Lead Exposure at California Hazardous Waste Sites

K. C. Gettmann, L. Nakayama Wong and M. J. Wade. *Dept Toxic Substances Control, Cal/EPA, Sacramento, CA.*

To evaluate lead exposure at California hazardous waste sites the Human and Ecological Risk Office (HERO) uses the CalEPA source-specific "benchmark change" lead criterion of 1 µg/dL (the estimated incremental increase in children's lead blood (PbB) reducing IQ by 1 point). HERO recommends use of LeadSpread 8 to evaluate lead exposure at our sites, and we are currently revising the model to use current state of the science. HERO is participating in US EPA's Beta Test Program for the All Ages Lead Model version 4 (AALMv4). We believe the AALMv4 will provide a scientifically sound base to compare with LeadSpread. HERO is testing how efficacious and flexible AALMv4 is with the range of site conditions and exposure scenarios seen at hazardous waste sites in California as compared to our LeadSpread 8 Model. At Site A, the maximum lead soil concentration is 388 mg/kg, the average concentration is 88 mg/kg, and the exposure scenario is a youth, exposed 3-days per week. Under the respective models' default parameters, the AALMv4 predicted a peak PbB of 0.71 µg/dL while LeadSpread 8 predicted a PbB of 2.2 µg/dL when exposed to 388 mg/kg lead in soil. Predicted PbB values of 0.16 µg/dL by AALMv4 and of 0.5 µg/dL by LeadSpread were obtained for exposure to the average lead concentration of 88 mg/kg. At Site B, the 95% upper confidence limit on the arithmetic mean (95%UCL) is 108 mg/kg, and the exposure is 7-days a week from birth to 7-years-old. The AALMv4 predicted a peak PbB of 1.19 µg/dL while LeadSpread 8 predicted a PbB of 1.4 µg/dL. The CalEPA screening value for lead in soil for residential land use is 80 mg/kg, while the US EPA value is 400 mg/kg. When evaluating a residential exposure scenario of 7-days a week from birth to 7-years-old, the AALMv4 predicated a peak PbB of 0.89 µg/dL and 4.3 µg/dL when children are exposed to 80 mg/kg and 400 mg/kg lead in soil, respectively. LeadSpread 8 predicted a PbB of 1 µg/dL and 5.2 µg/dL under the same conditions. Given the wide differences in the way the two models are constructed, the predicted PbB results appear to be comparable.

PS 2237 Physiologically Based Pharmacokinetic Modeling of the Reduction of Hexavalent Chromium in the Gastrointestinal Tract of Mice, Rats, and Humans

C. R. Kirman[1], D. M. Proctor[4], M. Suh[4], L. C. Haws[3], M. A. Harris[2] and S. M. Hays[1]. *[1]Summit Toxicology, LLP, Orange Village, OH; [2]ToxStrategies, Katy, TX; [3]ToxStrategies, Inc., Austin, TX and [4]ToxStrategies, Inc., Mission Viejo, CA.*

In an effort to extend previous modeling work, data from a series of *ex vivo* experiments in human gastric fluid (GF) samples were used to update the PBPK model for hexavalent chromium [Cr(VI)]. The new data address previously identified data gaps and limitations with respect to (1) the reduction of CrVI in fed human GF samples; (2) the number of CrVI reduction pools present in human fed, fasted, and proton pump inhibitor (PPI)-user GF samples; and (3) an appropriate form for the pH-dependence of CrVI reduction rate constants. The revised reduction model describes the Cr(VI) reduction by three pools (pool 1 = fast reaction with low capacity; pool 2 = slow reaction with higher capacity; pool 3 = very slow reaction with higher capacity), using pH-dependent rate constants. In addition to incorporating an updated reduction model, a number of refinements were made including: (1) characterization of drinking water exposure events in rodents over the course of a day (2-12x during day; 21-30x during night); (2) characterization of diurnal variation of gastric pH in rodents; and (3) separate characterization of human reduction capacities during fed (~28±29 mg/L) and fasted states (~16±21 mg/L). The results indicate that human gastric samples, like those collected from rats and mice, contain multiple pools of reducing agents; and low drinking water concentrations of CrVI (<0.7 mg/L) are reduced more rapidly than are high concentrations (>0.7 mg/L). The data and revised modeling results herein provide improved characterization of Cr(VI) gastric reduction kinetics and pharmacokinetic modeling for estimating target tissue exposure to Cr(VI) and provide a more robust characterization of inter-individual variation. The implications of the revised PBPK model for human health risk assessment are discussed.

PS 2238 A Comprehensive Physiologically Based Pharmacokinetic Knowledgebase and Web-Based Interface for Rapid Model Ranking and Querying

J. Lu[3], M.-R. Goldsmith[1], C. Grulke[2], D. Chang[1], J. Leonard[3], E. Hypes[3], M. Fair[3] and C. Tan[3]. *[1]Chemical Computing Group, Montreal, QC, Canada; [2]Lockheed Martin, Research Triangle Park, NC and [3]US EPA, Research Triangle Park, NC.*

Published physiologically based pharmacokinetic (PBPK) models from peer-reviewed articles are often well-parameterized, thoroughly-vetted, and can be utilized as excellent resources for the construction of models pertaining to related chemicals. Specifically, chemical-specific parameters and *in vivo* pharmacokinetic data used to calibrate and evaluate these published models can act as valuable starting points for model development of new chemicals with similar molecular structures. A knowledgebase for published PBPK-related articles was compiled to support PBPK model construction for new chemicals based on their close analogues within the knowledgebase, and a web-based interface was developed to allow users to query for those close analogues. A list of 689 unique chemicals and their corresponding 1751 articles was created after analysis of 2,245 PBPK-related articles. For each model, the PMID, chemical name, major metabolites, species, gender, life stages and tissue compartments were extracted from the published articles. PaDEL-Descriptor, a Chemistry Development Kit based software, was used to calculate molecular fingerprints. Tanimoto index was implemented in the user interface as a measurement of structural similarity. The utility of the PBPK knowledgebase and web-based user interface was demonstrated using two case studies with ethylbenzene and gefitinib. In both case studies, new PBPK models performed with a higher efficiency and achieved better predictive ability when using parameters based on

close analogues rather than those of non-analogues. Our PBPK knowledgebase is a novel tool for ranking chemicals based on similarities to chemicals associated with existing PBPK models and for rapid accessing of model-constraining publications. Disclaimer: This abstract has been cleared by the US EPA but solely expresses the view of the authors.

2239 Application of Physiologically-Based Pharmacokinetic Modeling to Explore the Role of Kidney Transporters in Renal Reabsorption of Perfluorooctanoic Acid in the Rat

R. R. Worley[1] and J. Fisher[2]. [1]*Division of Community Health Investigations, CDC/ATSDR, Atlanta, GA and* [2]*National Center for Toxicological Research, US FDA, Jefferson, AR.*

Renal elimination and clearance of perfluorooctanoic acid (PFOA) from the serum exhibit pronounced sex differences in the adult rat. The literature suggests that this is largely due to hormonally regulated expression of organic anion transporters on the apical and basolateral membranes of the proximal tubule cells that facilitate reabsorption of PFOA from the filtrate into the blood. Previously developed PBPK models of PFOA exposure in the rat have not been parameterized to specifically account for transporter mediated renal elimination. The model presented here explores the role of Oat1, Oat3, Oatp1a1 in sex-specific renal reabsorption and excretion of PFOA in adult male and female rats. Descriptions of the kinetic behavior of these transporters were extrapolated from *in vitro* studies and the model was used to simulate time-course serum, liver, and urine data for IV and oral exposures in the male and female rat. Predicted concentrations of PFOA in the liver, serum, and blood showed good agreement with experimental data for both male and female rats indicating that physiological descriptions of transporter-mediated renal reabsorption can successfully predict sex-dependent excretion of PFOA in the rat. This study supports the hypothesis that sex-specific serum half-lives for PFOA are largely driven by transporter expression in the kidney and contributes to the development of PBPK modeling as a tool for evaluating the role of transporters in renal clearance. Efforts are ongoing to apply a similar technique in the development of a PBPK model for human PFOA exposure, which will allow for improved evaluation of biomonitoring data.

2240 Evaluation of the Short-Term Clearance of Perflourooctanoic Acid (PFOA) in a Clinical Trial Using a PBPK Model and Markov Chain Monte Carlo Analysis

J. Campbell[4], B. Allen[2], G. Olsen[5], C. Elcombe[1], E. Doyle[1], J. Evans[3] and H. Clewell[4]. [1]*CXR Biosciences, Dundee, United Kingdom;* [2]*Independent Consultant, Chapel Hill, NC;* [3]*The Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom;* [4]*Center for Human Health Assessment, The Hamner Institutes, Research Triangle Park, NC and* [5]*Medical Department/Epidemiology, 3M, St. Paul, MN.*

Several studies have estimated a terminal half-life for the urinary clearance of perfluorooctanoic acid (PFOA) in the range of 2-4 years; however, the short-term clearance of PFOA has not been previously reported. In this study, single- and repeated-dose pharmacokinetic data from a human clinical trial with 50 patients being treated for cancer (CXR database) was analyzed using a physiologically based pharmacokinetic (PBPK) model in a Markov chain Monte Carlo (MCMC) analysis. The MCMC approach allowed for incorporation of prior knowledge about PFOA kinetics and a hierarchical structure accounting for inter-individual variability. The median age of the patients in the trial was 63 with a minimum of 36 and maximum of 74. The half-life in these patients had a mean of 0.13 yrs with a range of 0.013 to 0.8 yrs, significantly shorter than estimates of the terminal half-life derived from chronic exposures. The relationship of PFOA dose and AUC was not suggestive of a significant departure from linearity for doses ranging from 50 to 1200 mg/day for up to 6 weeks. Only a small portion of the variance in AUC was accounted for by GFR, suggesting that filtration is not as influential as reabsorption in the short-term clearance of PFOA. Based on this finding, the calibration was designed to refine the most sensitive parameters in the model, including the maximum velocity and affinity constant for renal resorption and the free fraction in plasma.

2241 Comparative Respiratory Toxicity of PM 2.5 to Human Lung Cells Cultured in Three Dimensions and in Monolayers

E. R. A. Ferraz[1,2], C. R. Rainho[1], A. S. Fernandes[1] and I. Felzenszwalb[1]. [1]*Department of Biophysics and Biometry, University of the State of Rio de Janeiro, Rio de Janeiro, Brazil and* [2]*Department of Pharmacy and Pharmacy Administration, Fluminense Federal University, Niteroi, Brazil.* Sponsor: D. Oliveira.

Several epidemiological studies have associated PM 2.5 with an increase in morbidity and mortality from cardiopulmonary diseases, which shows the importance of carrying out a risk assessment from exposure to this xenobiotic. In this context, given the growing effort to replace the use of animals in research, cytotoxicity induced by PM 2.5 was investigated using A549 cells cultured in three dimensions (3D), an alternative method to the use of animal models that has proved promising, and cultured in monolayers for comparison. PM 2.5 was collected on Brazil Avenue, Rio de Janeiro, Brazil, every month from November 2010 to May 2011, except March, and analyzed for inducing cytotoxicity in A549 cells whether by affecting the mitochondrial dehydrogenase activity using WST-1 assay, or cell membrane integrity through LDH assay. PM 2.5 collected in November poorly induced cytotoxicity to A549 cells. This effect was observed only after 72 hours of incubation and just in 3D cell culture (WST-1 assay) and in both culture systems (LDH assay). However, PM 2.5 collected on all other months induced high cytotoxicity by affecting the mitochondrial dehydrogenase activity and by disrupting the plasma membrane. It is so important to point out that with the exception of PM collected in April the PM collected in all other months induced greater toxicity in cells grown in monolayers system, in which cells grow adhered to a rigid plastic surface in a two-dimensional geometry, than in 3D cell culture. The present results show that cell behavior varies according to the type of culture system used, and since the 3D cell culture mimics the architecture of the *in vivo* tissue more reliably, it is suggested that data from 3D studies are closer to the true exposure conditions, which aids in risk assessment on exposure to MP with more robust data than those obtained in traditional culture system. Acknowledgment: FAPERJ, CNPq

2242 Concentrations of Individual Fine Particulate Matter Components in the United States Around the 4th of July

E. Chan[2,3], A. Dickerson[1,3] and B. Buckley[2]. [1]*Integrated Risk Information System, Office of Research and Development, US EPA, Durham, NC;* [2]*National Center for Environmental Assessment, Office of Research and Development, US EPA, Durham, NC and* [3]*Oak Ridge Institute for Science and Education, Oak Ridge, TN.*

The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Firework displays emit particulate matter (PM) air pollution, the toxicity of which varies by size and composition. No studies to date have used national, multi-year air quality monitoring data to determine which individual PM components with a nominal mean aerodynamic diameter less than or equal to 2.5 µm (PM2.5) increase due to 4th of July firework displays. To evaluate this, we compiled and analyzed daily average PM2.5 air quality data collected from over 400 monitors positioned across the United States (US) over the years 2000-2014. By collapsing all individual daily arithmetic mean PM2.5 concentrations recorded and viewing these data points over time, we observed sharp and significant ($p \leq 0.001$) increases in the concentrations of the firework-related chemicals barium, copper, magnesium, potassium, and strontium on the 4th and 5th of July, with the highest concentrations of these components occurring on the 4th. In fact, the average national PM2.5 concentration of potassium on July 4th was 15-times greater than that over all other monitored days (0.799 v 0.053 µg/m3). When comparing the national average PM2.5 component concentrations on July 4th to the combined average over July 1st, 2nd, 3rd, 5th, and 6th, there were also small, but statistically significant, increases on July 4th of the firework-related components antimony, aluminum, carbon, sodium, sulfur, titanium, and zinc ($p \leq 0.05$), but not for cesium, chlorine, iron, phosphorous, and sodium. These findings provide information relevant to human exposures to potentially hazardous firework components in the US on and around the 4th of July that could be used in air quality models and human health risk assessments.

2243 ROS Species Present on PM Are Associated with Biological Changes in Humans Exposed to Fine + Ultrafine CAPS

R. Devlin[1], V. Verma[2], A. Rappold[1], H. Tong[1] and R. Weber[2]. *[1]US EPA, Research Triangle Park, NC and [2]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA.*

Several studies have shown that inhalation of PM can induce oxidative stress on target organs or tissues. However, it is not clear whether that stress is caused by ROS generated from particles or by the ability of inhaled particles to contribute to the formation of endogenous intracellular or extracellular oxidative stress. To assess the ability of particle-associated ROS to cause biologic changes in humans, DTT activity (a measure of ROS) was quantified in water soluble and methanol soluble material extracted from Teflon filters that captured PM mass during a study in which humans were exposed to concentrated ambient air particles (CAPS). Twenty-nine volunteers were exposed for two hours on two occasions: once to clean air and once to fine + ultrafine CAPS. Linear mixed effects models with subject specific random intercept were used to test changes in effects between the CAPS and air exposures. Robust associations were observed between both PM mass and particle number and changes in endothelial cell function, HRV, cardiac repolarization, blood lipids, and cellular and soluble vascular markers. Water soluble PM was extracted from 47 mm Teflon filters collected during the CAPS exposures by sonication in deionized water for 1 hr. A portion of the extract was used to measure total DTT content. A portion was passed through a C-18 column, which binds hydrophobic material. The material passing through the column (hydrophilic material) was assayed for DTT activity. The hydrophobic DTT activity was determined by difference. A portion of the filter was also extracted in methanol. We compared the association between particle generated ROS and health end points. Changes in endothelial function were associated with total DTT, hydrophilic DTT and hydrophobic DTT activity. Changes in cardiac QT interval were associated with the total and hydrophilic DTT fraction. Changes in blood lipids and cells were associated with the methanol DTT fraction. We conclude that particle-associated DTT activity is strongly associated with biologic end points altered by PM exposure and that different DTT fractions are associated with different groups of biological end points. This abstract of a proposed presentation does not represent EPA policy.

2244 Temperature and Calcium Dependent Activation of TRPM8 by Particulate Materials

S. K. Marcus, Z. Lu, E. G. Romero, C. E. Deering-Rice and C. A. Reilly. *Pharmacology & Toxicology, University of Utah, Salt Lake City, UT.*

Transient receptor potential melastatin-8 (TRPM8) and an N-terminally truncated variant (ΔM801) expressed by human lung epithelial cells are activated by selected particulate materials (PM). We investigated the mechanism of TRPM8 activation, and the consequences in the lungs of mice. TRPM8 is cold-sensitive. Cold media did not activate TRPM8, but responses to PM, menthol, and icilin were increased when applied as cold solutions. Mutants of the high-affinity menthol and icilin binding sites (Y745H and L1009R), and the ΔM801 variant were functionality compared to wild-type TRPM8 using a Ca++ flux assay. The Y745H, L1009R, and ΔM801 variant were activated by PM, but not icilin or low concentrations of menthol. Constituents of the PM coal fly ash (CFA) were identified using scanning-electron microscopy and energy dispersive X-ray spectroscopy. An abundance of calcium nanoparticles was observed. Multiple nanoparticles were tested as agonists, but only calcium oxide and calcium phosphate were agonists. The amount of free calcium associated with CFA and calcium nanoparticles was proportional to TRPM8 activation. Dialyzing CFA and calcium oxide nanoparticles eliminated TRPM8 activation, indicating calcium ions released from the particles caused TRPM8 activation. CFA induced multiple pro-inflammatory genes in mouse lungs, which was attenuated in trpm8-/- mice. These findings advance our understanding of specific interactions that occur between PM and lung cells by identifying TRPM8 as a mediator of certain toxicological phenomena caused by CFA. This study provides new insights into mechanisms by which other calcium-containing PM and inhaled materials might cause lung irritation and toxicity.

2245 Regulatory T Cells and IL10 Suppress Pulmonary Host Defense During Early Life Exposure to Radical Containing Ultrafine Particulate Matter

S. Jaligama, N. Yadav, J. Minso, G. I. Lee and S. A. Cormier. *Pediatrics, University of Tennessee Health Science Center, Memphis, TN.*

Exposure to elevated levels of particulate matter (PM) is associated with adverse respiratory health in infants and increased risk of morbidity and mortality due to respiratory tract viral infections. Our previous studies demonstrated that combustion derived PM (CDPM) induced immunosuppression allowing for enhanced influenza disease severity and mortality in neonatal mice. Current studies are focused at understanding the mechanism of this immunosuppression. Neonatal mice (3 days old) were acutely exposed to DCB230, a CDPM, and infected with influenza virus. Pulmonary T cell phenotypes including regulatory T cells (Tregs) were analyzed by flow cytometry. A significant increase in pulmonary Tregs and immunosuppressive cytokines IL10 was observed in PM exposed and influenza infected (DCB/Flu) mice, which peaked at 10 days post exposure compared to air exposed and influenza infected mice (Air/Flu). This coincided with decreased IFNγ positive T helper 1(Th1) and cytotoxic T cell (Tc1) responses that play an important role in protecting against influenza infection. Further, DCB exposed mice that were depleted of Tregs by anti-CD25 antibody (PC61) and infected with influenza (PC61/DCB/Flu) exhibited significant increases in both Th1 and Tc1 cells compared to DCB/Flu mice. Adoptive transfer of Tregs from DCB exposed mice into air exposed mice prior to infection with flu suppressed Th1 and Tc1 responses. To elucidate the role of IL10, air exposed neonatal mice were treated with recombinant IL10 (rIL10) and subsequently infected with influenza (rIL10/Flu). rIL10/Flu mice had increased incidence of morbidity and pulmonary viral load compared to Air/Flu mice. Furthermore, IL10 deficient mice (IL10KO) exposed to PM and infected with influenza (IL10KO/DCB/Flu) exhibited significant reductions in pulmonary viral load and inflammation indicative of enhanced protection against influenza. Together, these studies demonstrate that PM induced Tregs and their effector cytokine, IL10, suppress adaptive T cell responses leading to increased influenza severity in infected neonatal mice.

2246 Enhanced Nitrative and Oxidative Dysfunction of Upper and Lower Respiratory Tissues in World Trade Center Dust Exposed Mice

M. Hernandez, A. Harrington, K. Galdanes, T. Gordon and L.-C. Chen. *Department of Environmental Medicine, New York University, Tuxedo, NY.* Sponsor: L.-C. Chen.

In the last fourteen years, workers and residents near the World Trade Center (WTC) site have been plagued by lower respiratory ailments similar to those who suffer from particulate matter (PM) exposure. This study evaluated intranasally administered WTCPM<53µm in upper and lower respiratory tissues and resultant nitrative and oxidative outcomes. Male C57Bl/6 mice were intranasally exposed to 6 doses of WTCPM ranging from 1mg/50µl to 31ug/50µl, with significant polymorphonuclear neutrophil (PMN) increases ranging from 20-30% in both nasal (NLF) and bronchoalveolar lavage fluids (BALF), in both singular and repeated exposures. Mice allowed a 90 day recovery did not elicit any BALF PMN increase. All BALF samples contained WTCPM<53µm retrieved particles, even after 90 days recovery. Pulmonary gene expression analysis found HO-1 and SOD-2 mRNA to be similarly expressed throughout the exposure matrix. Initially, both genes were not significantly altered in single exposure scenarios, but were significantly downregulated (4 and 5 fold, respectively) after multiple exposures, and back to control levels or above (1.33- 2.7 fold) 90 days post treatment. BALF nitric oxide (NO) levels were found to be significantly decreased (20-92%) in all singular exposures of WTCPM<53µm as compared to controls, but were found to return to near control levels in animals exposed multiple times. NO concentrations in 90 days recovery BALF were found to contain a combination of decreased (36-60%) and increased (90%) NO levels. HO-1 and Nrf2 gene expression in olfactory mucosa was found to be moderately upregulated (1.4 fold), while SOD-2 was found to be significantly downregulated (2.3 fold) after multiple exposures and after 90 days recovery. In a co-exposure scenario, mice were exposed to 25µg/ 50µl of PM2.5, 63µg or 125µg/50µl of NIST diesel, singularly or in conjunction with 31µg/50µl of WTC PM<53µm. WTCPM was found to significantly potentiate PMN influx in BALF (15-27%) and NLF (10%) of co-exposed mice. These data suggest WTCPM exposure enhances nitrative and oxidative dysregulation in pulmonary and nasal tissues, as well as potentiates inflammation based mechanisms. More importantly, WTC co-exposure scenarios may further potentiate environmentally induced injuries brought about by repetitive insult from ambient pollutants. [Supported by NIH F31 ES 025591]

2247 Transcriptome Analysis Reveals Chronic Ailment Progression From Fine, Coarse, and Supercoarse World Trade Center Particles on Exposed Rat Lung and Liver

M. Marshall, L. Horton, M. Sisco, C. Prophete, H. Sun, J. Zelikoff, M. Cohen and L.-C. Chen. *Environmental Medicine, New York University School of Medicine, Tuxedo, NY.*

On September 11, 2001 and subsequent days following the attack on the World Trade Center (WTC) buildings, a dense concentration of particles filled the air comprised of an amalgamation of debris from the fallen buildings, ranging in size from fine, coarse, and predominantly supercoarse (10–53 µm) particles. Fourteen years later, chronic health effects have become apparent in First Responders (FR) exposed to WTC dust over the first 72 hours due to the unique exposure scenario in which mouth breathing was prevalent. This study sought out to investigate the potential effects of WTC dust by exposing male F344 rats intratracheally to WTC_PM≤53, which properly mimicked mouth-breathing by FR. Each rat received a ~33 mg/m3 concentration of WTC dusts for 2-hrs for 2 consecutive days and sacrifices occurred 7 days and 360 days post exposure. Lungs and liver underwent RNA Sequencing analysis to show differentially-expressed genes (DEGs). Ingenuity Pathway Analysis revealed disparate differences in DEGs on 7d and 360d related solely to WTC dust exposure. Results showed an activation of edema (z-score=3.98), atherosclerosis (z-score=2.241), and atherosclerotic lesions (z-score=2.04) in 360d lung samples, as well as a decrease in left ventricle dilation (z-score=-2.588). In 360d liver samples, proliferation of hepatic stellate cells (HSCs) became activated (z-score=2.533), while apoptosis of HSCs decreased (z-score=-2.183). Also, liver samples from 360d showed an activated inflammatory response (z-score=3.761). Most significant 7d liver and lung effects appeared to be transient; however, these outcomes probably hinder pathways that manifest into 360d injuries. This study demonstrates WTC dust ability to exacerbate injury with time and offers possible insight into the chronic health effects experienced by FR.

2248 Pulmonary and Cardiovascular Effects in Mice After Intratracheal Instillation of Particles from Burning Candles in Mice

P. Moller[2], A. Skovmand[2], A. C. Gouveia[2], I. K. Koponen[1], S. Loft[2] and M. Roursgaard[2]. [1]*The National Research Centre for the Working Environment, Copenhagen O, Denmark and* [2]*Department of Public Health, University of Copenhagen, Copenhagen K, Denmark.*

Burning candles emit large amounts of particles, which deposit in the airways. This can be an important source of particles in dwellings or public spaces such as restaurants. It is well-known that other types of combustion particles cause damage to lung cells and affects the cardiovascular system. We collected particles from burning candles with a high-volume electrostatic field sampler. C57BL/6 mice were exposed to suspensions of particles by intratracheal instillation (0.5 or 5 mg/kg body weight) and sacrificed 24 hours post-exposure. The particle size in suspension medium (nanopure water with 0.1% Tween) was 155 nm, which was similar to benchmark diesel exhaust particles (SRM2975, 167 nm, assessed using Nanosight Tracking Analysis). The high dose exposure to candle light combustion particles was associated with increased influx of leukocytes (7.9-fold, 95% confidence interval: 5.7-10.1 fold, increased as compared to vehicle control), protein concentration (2.1-fold, 95% CI: 1.8-2.4) and lactate dehydrogenase level (6.4-fold, 95% CI: 5.0-7.9) in bronchoalveolar lavage fluid. Candle light combustion particles produced the same or higher level of toxicity in the lungs as compared to diesel exhaust particles. Nevertheless there were unaltered levels of DNA strand breaks and oxidatively damaged DNA in lung tissue of mice after exposure to candle light combustion particles. The lack of genotoxicity was verified in cultured A549 lung epithelial cells. Exposure of particles to atherosclerosis-prone apolipoprotein E knockout mice on Western-type diet (0.5 or 5 mg/kg once a week for five weeks) was not associated with pulmonary inflammation or toxicity. There was only slightly increased level of atherosclerotic plaques in aorta in the group of mice that was exposed to candle light combustion particles. In conclusion, pulmonary exposure to candle light combustion particles is associated with pulmonary inflammation and cytotoxicity, whereas the current exposure model did not give rise to genotoxicity in lung tissue or systemic effects in terms of plaque progression.

2249 Abstract Withdrawn by Author.

2250 Fine Particulate Matter (PM2.5) Exposure and the Cardiometabolic Effects in Circadian Dyssynchrony

P. Haberzettl, A. Bhatnagar and D. J. Conklin. *University of Louisville, Louisville, KY.*

Recent human and animal studies suggest that either exposure to ambient air fine particulate matter (PM2.5) or a disturbed circadian rhythm (dyssynchrony, e.g. light pollution, shift work) contribute to the rapidly evolving epidemics of obesity and diabetes. However, the cardiometabolic effects of combining PM2.5 exposure and dyssynchrony are unknown. Thus, we exposed mice under light-induced dyssynchrony (18h light:6h dark, disturbed circadian rhythm) to concentrated ambient PM2.5 (CAP, 30 consecutive days, 6h/d) and examined cardiometabolic changes. CAP exposure enhanced the body weight of mice in dyssynchrony but not in mice kept on a regular light cycle (12/12h light/dark). Furthermore, CAP exposure in mice with light-induced dyssynchrony increased systemic glucose intolerance and insulin resistance - determined by glucose tolerance test and HOMA-IR scores - but not in mice kept on a regular light cycle. Increased systemic insulin resistance in CAP-exposed mice kept on a disturbed light cycle was accompanied by decreased insulin signaling - insulin-induced Akt phosphorylation - in the skeletal muscle that was not observed in mice on a regular light cycle. Likewise, CAP exposure induced hepatic and cardiac insulin resistance in dyssynchrony. In contrast, CAP exposure had no effects on insulin signaling in the adipose tissue of mice kept on a disturbed or regular light cycle. Collectively, our results suggest that CAP exposure provokes systemic insulin resistance in dyssynchrony possibly by impairing insulin signaling in skeletal muscle and liver thereby increasing the risk for the development of T2D after exposure to elevated levels of PM2.5.

2251 Chemical Composition Determines TRPA1 and Other Cellular Responses to Diesel Exhaust Particulate Materials

Z. Lu, C. E. Deering-Rice and C. A. Reilly. *Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.*

Diesel exhaust particulates (DEP) are a common source of fine-particulate air pollution (PM). Inhalation of DEP causes pulmonary irritation and may contribute to pulmonary inflammation, and exacerbation of chronic pulmonary diseases. Transient receptor potential ankyrin-1 (TRPA1) is activated by DEP from various sources: a poorly functioning on road "black smoker" truck, an industrial fork lift "NIST SRM2975," material recovered from regenerated DEP filters, and DEP collected at an emissions "IM" station. Potency was related to the percentage of extractable TRPA1 agonists; both electrophiles and non-electrophiles, measured using a TRPA1 over-expressing HEK-293 cell model. The relationship between chemical composition and TRPA1 potency in primary human pulmonary fibroblasts (IMR90 cells), which are one of the few primary lung cell lines that express TRPA1, was also assessed. Furthermore, the relationship between DEP composition, potency at TRPA1, and measures of lung cell toxicity was explored. Ca++ flux in IMR90 cells caused by "black smoker" and "IM" DEP was comparable on a per-mass basis, and these were the most potent TRPA1 agonists, followed by NIST and filter DEP. Taqman Real-Time PCR analysis demonstrated induction of mRNA for the pro-inflammatory cytokine IL-8; the least potent DEP, filter DEP, and it's major chemical component 2,4-ditert butylphenol, induced IL-8 to the greatest extent. The relative abundance of neutrophils recovered in BAL from DEP-treated mice agreed the qPCR results for IL-8. This study demonstrates that TRPA1 is differentially activated by qualitatively and quantitatively different DEP via multiple mechanisms, but that potency as a TRPA1 agonist does not necessarily predict common cellular responses to DEP; the chemical composition is important. It appears that both general and specific cellular responses occur in response to different DEP, with the overall response perhaps being "fine-tuned" by TRPA1. Support: ES017346

2252 Zebrafish Locomotor Responses Demonstrate Irritant Effects of Fine Particulate Matter Sources and a Role for TRPA1

J. Stevens[2], D. DeMarini[2], M. S. Hazari[2], D. L. Hunter[2], S. Padilla[2] and A. K. Farraj[2]. *[1]Environmental Public Health Division, US EPA, Research Triangle Park, NC and [2]Integrated Systems Toxicology, US EPA, Research Triangle Park, NC.*

Fine particulate matter (PM) air pollution is a complex mixture of chemicals, the composition of which is determined by contributing sources, and has been linked to cardiopulmonary dysfunction. These effects stem in part from the irritating properties of PM constituents, which often elicit pain and inflammatory responses via activation of the chemosensing transient receptor potential (TRP) cation channel TRPA1. Zebrafish larvae sense a variety of chemical stimuli, the behavioral responses to which can be easily measured in routine assays. The purpose of this study was to examine the utility of the zebrafish as a sentinel species to assess PM sources for their capacity to trigger irritant responses. We hypothesized that exposure of zebrafish embryos to an organic fraction of diesel exhaust particulate matter (ODEP), a PM rich in pro-oxidant polyaromatic hydrocarbons, would elicit concentration-dependent irritant responses. In addition, given that zebrafish express TRPA1 on their skin, we hypothesized that the ODEP-induced irritant responses in zebrafish would be mediated by activation of TRPA1. Six day-old zebrafish (n=24/experimental condition) were acutely exposed to ODEP (8 to 40 µg/ml) and activity was assessed in the dark at 26.0°C for 30 minutes using video tracking software. Activity was also measured in ODEP-exposed (28 µg/ml) fish pretreated with a TRPA1 antagonist (HC 030031, 50 µM). Acute ODEP exposure caused marked concentration-dependent hyperactivity. Furthermore, pretreatment with the TRPA1 antagonist blocked the ODEP-induced hyperactivity response. Taken together, the dynamic range of the assay and the mediation of responses by mechanisms analogous to those in mammals support zebrafish as a useful model to assess PM sources for their ability to elicit irritant-responses (This abstract does not reflect US EPA policy).

2253 Immunologic Effect of Ozone and PM2.5 from Mexico City in a Guinea Pig Asthma Model

L. I. Garcia-Alonso[2,4], C. I. Falcon-Rodriguez[4], A. Osornio-Vargas[3], N. Manzano-Leon[1], I. Rosas-Perez[2], A. De Vizcaya-Ruiz[5] and P. Segura-Medina[4]. *[1]INCAN, Mexico City, Mexico; [2]Centro de Ciencias de la Atmósfera, UNAM, Mexico City, Mexico; [3]Deparment of Pediatrics, University of Alberta., Edmonton, AB, Canada; [4]Departamento de Investigacion en Hiperreactividad bronquial, INER, Mexico City, Mexico and [5]Departamento de Toxicologia, CINVESTAV, Mexico City, Mexico; [6]Facultad de Ciencias, UNAM, Mexico City, Mexico; [7]Hiperreactividad bronquial, INER, Mexico City, Mexico.* Sponsor: A. de Vizcaya-Ruiz.

Air pollution is a public health issue, particularly in Mexico City where we found two persistent pollutants: Ozone (O3) and PM2.5 (particulate matter) that use to exceed daily their norm. Some studies report that exposure to these pollutants can induce asthma in both, animal models, and humans. Our aim was to evaluate the immune response after the exposition to O3 and PM2.5 in an allergic sensitized guinea pig model, in cold-dry season (November-February), since during this period, the pollutants use to persist longer in the atmosphere. Guinea-pigs were sensitized (day 1) with ovalbumin (OVA) plus AlOH3 as adjuvant, and exposed to filtered air, or PM2.5 (4h/day) and/or Ozone (1h/day) during 9 days using an Aerosol Concentrator Systerm at CINVESTAV-Zacatenco. We made dose-response curves to Histamine using barometric plethysmography and 24-hours later Broncho-alveolar Lavage (BAL) was performed to measure by ELISA assay: CC16, IL4, IL10, IL 17, TNF-α and INF-γ. Histamine curves demonstrated airway hyperresponsiveness in asthmatic animals. The levels of the epithelial damage biomarker: CC16 increased in all groups. The inflammatory response to TNF-α increase, especially in the group exposed to PM2.5. Anti-inflammatory response of IL-10 decreases in all groups. Also TH1 and TH2 cytokines responses shows: a significative increase of IL-4 levels after O3 and PM2.5 inhalation. Furthermore, INF-γ had a significant arose in the asthmatic group exposed just to PM2.5. Our results suggest that inflammatory mechanisms to these pollutants are different, despite the asthmatic inflammation process. It will be important to evaluate the inflammation mechanisms and their temporality of the pollutants in the future. Support: SECITI 014/2014.

2254 Pulmonary Inflammatory Responses to Acute Meteorite Dust Exposures - Implications for Human Space Exploration

A. D. Harrington[3,5], F. M. McCubbin[1], J. Kaur[5], A. Smirnov[2,5], K. Galdanes[3], M. A. Schoonen[4,5], L.-C. Chen[3], S. E. Tsirka[5] and T. Gordon[3]. *[1]NASA Johnson Space Center, Houston, TX; [2]Earth and Marine Sciences, Dowling College, Oakdale, NY; [3]Environmental Medicine, New York University School of Medicine, Tuxedo, NY; [4]Environmental Sciences, Brookhaven National Laboratory, Upton, NY and [5]Geosciences, Stony Brook University, Stony Brook, NY; [6]Pharmacological Sciences, Stony Brook University, Stony Brook, NY.*

New initiatives to begin Lunar and Martian colonization within the next few decades are illustrative of the resurgence of interest in space travel. However, as with all exploration, there are risks. One of NASA's major concerns with extended human space exploration is the inadvertent and repeated exposure to unknown dust. This highly interdisciplinary study evaluates the relative toxicity of six meteorite samples representing either basalt or regolith breccia on the surface of the Moon, Mars, and Asteroid 4Vesta. Terrestrial mid-ocean ridge basalt (MORB) is also used for comparison. All material is fully characterized and evaluated for geochemical reactivity (e.g. iron solubility and acellular reactive oxygen species (ROS) generation). Both *in vitro* and *in vivo* toxicological techniques are used to determine the pulmonary inflammation caused by acute exposure. The MORB demonstrated higher geochemical reactivity than most of the meteorite samples but caused the lowest acute pulmonary inflammation (API). Notably, the two Martian meteorites generated some of the highest API but only the basaltic sample is significantly reactive geochemically. Furthermore, while there is a correlation between a meteorite's soluble iron content and its ability to generate acellular ROS, there is no direct correlation between a particle's ability to generate ROS acellularly and its ability to generate API. However, assorted *in vivo* API markers did demonstrate strong positive correlations with Fenton metal content and the ratio of Fenton metals to silicon. In summary, this comprehensive dataset allows for not only the toxicological evaluation of celestial materials but also clarifies important correlations between geochemistry and health.

2255 Differences in Responses to Diesel Exhaust Particles from Asthmatic and Non-Asthmatic Bronchial Epithelial Cells

L. B. Mendez, W. Torres-Rivera and C. Torres. *School of Science and Technology, Universidad del Este, Carolina, PR.*

It is well known that asthmatic individuals are at a greater risk for the respiratory effects of particle pollution. However the molecular mechanisms that render asthmatic individuals more susceptible to the adverse effects of particle pollution have not been fully elucidated. Therefore the main objective of this study is to determine exposure-dose relationships of PM cytotoxic effects in asthmatic and non-asthmatic bronchial epithelial cells in order to gain insights about possible mechanisms of susceptibility. Primary human bronchial epithelial cells derived from asthmatic (AHBE) and non-asthmatic (NHBE) individuals were acutely exposed (20 min to 480 min) to different concentrations of diesel exhaust particles (DEP; 0 to 25 µg/mL). Physiologically relevant concentrations were calculated with the multiple-path particle dosimetry model using the human age-specific symmetric model for ages 8 to 9. Multiple endpoints of cell viability and cytotoxicity, including metabolic activity and apoptosis, were measured within the same well 24 hours after initial exposure. Dose-response analysis revealed that AHBE cells were more prone to undergo into apoptosis than NHBE cells. In addition, AHBE cells were more metabolically active than NHBE, as assessed by the alamar blue assay. A positive correlation (r = 0.84 to 0.94) was observed between metabolic activity and caspase-3 activity in AHBE cells suggesting that cellular metabolism impairment might render AHBE more susceptible to the effects of DEP. This study shows that asthmatic cells respond different than non-asthmatic cells when exposed to the same doses of DEP, a main constituent of urban particle pollution. Differences in cellular responses might explain the susceptibility of asthmatic individuals to the adverse respiratory effects of particle pollution. Overall, our results provide information about the biological mechanisms underlying the adverse effects of particle pollution in asthmatic individuals.

2256 Size-Resolved Oxidative Potential of Traffic-Related Particles

R. V. Luna-Serrano, E. D. Gonzalez-Figueroa, W. Torres-Rivera and L. B. Mendez. *School of Science and Technology, Universidad del Este, Carolina, PR.*

Asthmatic children are at a greater risk for adverse respiratory effects due to particle pollution exposure, especially those living in urban areas next to a busy road and/or highway. The oxidative potential (OP) of particles, which is defined as its capacity to oxidize target molecules, has been proposed as toxicologically relevant metric for particulate matter (PM) since it is better related to their exerted biological effects. Therefore the objectives of this study are to: 1) assess traffic-related OP and 2) evaluate the size distribution of traffic-related particles based on their OP. In order to do this we sampled for two consecutive weeks in the parking lot of an elementary school located near two main sources of traffic-related pollution. Samples were collected with an 8-stage cascade impactor and particles were extracted from the filters with water by sonication for 1 hour. The OP for particles deposited in each stage was determined with the DTT assay. The mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) were calculated using the log-probability method to describe the particle size distribution based on its oxidative characteristics. The mean OP measured was 23.41 nmoles of consumed DTT/m3 (95% CI 5.82-40.99). Differences between OP and size fraction were observed. Mostly particles with higher OP were more prevalent within the fine fraction (MMAD ~ 1.4 µm and GSD ~ 3.3 µm). On occasion's particles in the submicron fraction accounted for more than 90% of the observed oxidative potential. This data will be used as complimentary information for exposure assessments to evaluate if OP measurements are associated with respiratory effects in asthmatic children.

2257 The Association Between Ambient PM2.5 Concentration and Rate of Asthma Hospitalizations in Louisiana, 2009

J. Ding, K. Gam and M. Lichtveld. *Global Environmental Health Sciences, Tulane University, New Orleans, LA.*

Asthma is a chronic disease that has been shown to disproportionately affect those with income disparities; Louisiana's poverty rate ranks second highest in the US. Previous epidemiological studies have linked ambient fine particulate matter to asthma hospitalizations (AH). Fine particulate matter 2.5 micrometers in diameter or less (PM2.5) is a mixture of tiny particles and droplets, often containing acids, organic chemicals, soil, and dust. The purpose of this community environmental health analysis is to determine the association between PM2.5 levels and the rate of AH in Louisiana in 2009. Air pollution data was collected from CDC Environmental Public Health Tracking Network (EPHT). Hospitalization data was collected from Louisiana EPHT. Data analysis was conducted using SAS 9.3. Bivariable analysis was conducted using Student's t-Tests. The relationship between PM2.5 and asthma was estimated using multivariable linear and logistic models that adjusted for exceedance of ozone, age, gender, median income, smoking, and urban/rural. In 2009 the mean PM2.5 level in Louisiana was 9.35 (SD: 0.69) µg/m3, and the parish PM2.5 levels ranged from 8.4 to 11.6 µg/m3. The average rate of asthma hospitalizations was 15.4 per 10,000 persons (SD: 6.72). The relationship between PM2.5 and asthma was estimated using multivariate linear and logistic models that adjusted for covariates such as ozone, age, gender, median income, smoking, and urban/rural. The logistic model showed no statistically significant association between PM2.5 levels and AH rates; the odds ratio was 3.73 (95% CI: 0.52, 26.98). PM2.5 monitors are concentrated in Southeastern Louisiana; 68.33% of Louisiana's population resides in a parish with a monitor. The number of monitors and number of parishes monitored vary by criteria pollutant. Future studies are needed to assess and develop methods for measuring air pollution in environmental epidemiological studies.

2258 Evaluation of Different Particle Size Quantification Instruments - Strengths/ Weaknesses and Characteristics for Consideration in Determining the Appropriate Technique

P. J. Kuehl, D. Kracko, H. Irshad, E. Yates and J. McDonald. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

The particle size distribution (PSD) of an aerosol is a key characteristic that must quantified for clinical and pre-clinical aerosols. These aerosols can be therapeutic and / or environmental in nature. They often include liquid/droplet, dry powders, and complex mixtures. The particle size distribution can be measured via a variety of different instruments that utilize different techniques to separate the aerosols by size and quantify the aerosols within these size ranges. Each instrument has advantages and disadvantages in their analysis/collection methods that must be considered prior to selection as the appropriate technique/instrument. This work reviewed several instruments that are commonly utilized and evaluated them with idealized, liquid, dry powder, and biological aerosols. The instruments included cascade impactors (next generation impactor, Anderson, Mercer, Viable and Multi-stage liquid impinger) that resolve aerosols based on inertial energy, the TSI Aerodynamic Particle Sizer (APS) that classifies particles based on time of flight, the GRIMM aerosol spectrometer that classifies particles via laser spectrometry. Each instrument was evaluated with monodisperse aerosols (oleic acid particles tagged with sodium fluorescein) at 2.5 µm Mass Median Aerodynamic Diameter (MMAD) and shown to be within 20% of each other. During analysis of a model engineered dry powder the measured PSD (MMAD) from the APS was 2.8 µm, 2.5 µm with the Mercer impactor and 3.7 µm with the GRIMM. Analysis of a model biologic aerosol (from aqueous media) the MMAD was measured with GRIMM (2.8 µm), the APS (2.5 µm) and the Mercer impactor (2.9 µm). Contributing to the measured differences is the fact that the APS can de-agglomerate dry aerosols and dry wet aerosols thereby decreasing the particle size. Cascade impactors are susceptible to particle 'bounce' resulting in a decreased particle size measurement. Additionally each instrument varies in its ability to be utilized under regulatory settings (GLP studies). These data provide a foundation for the selection of the appropriate device for PSD measurement.

2259 Investigation of Acute Pulmonary Deficits Associated with Biomass Fuel Cookstove Emissions in Rural Bangladesh

J. Angermann[3], D. Medgyesi[3], J. Parks[3] and H. Holmes[3]. [1]*University of Nevada, Reno, NV;* [2]*Atmospheric Science Program, Department of Physics, University of Nevada, Reno, NV and* [3]*School of Community Health Sciences, University of Nevada, Reno, NV.*
Sponsor: J. Angermann.

The use of solid biomass fuels in cookstoves has been associated with long term chronic health impacts that disproportionately affect women worldwide. The immediate effects on acute pulmonary function, however, have not been investigated. This study measured the respiratory impact of exposure to biomass fuel emissions for 12 females aged18-65 in rural Bangladesh. Solid fuel stoves that use wood, litterfall, and cow dung are common in rural Bangladesh and cooking is done in a separate room from the main house. Pulmonary function was measured with spirometry before and during cooking to assess changes in respiratory function associated with exposure to cookstove emissions. Specifically, the forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1 over FVC ratio, and peak expiratory flow (PEF) were compared. Cookstove emissions were characterized using continuous measurements of particulate matter (PM) concentrations at a 1 second time resolution. Exposure metrics were calculated from the measured PM data to estimate the acute exposure to cookstove emissions for each study participant. Exposure estimates include 3-minute maximum, 30-minute average, and the time weighted average PM concentration for the cooking time period. Litterfall (i.e., leaves and twigs) and household material waste (i.e., paper and plastic wrappers) emissions have the highest 3-minute maximum PM concentration. Larger or open cooking areas have lower PM concentrations due to increased ventilation. Participants that have been preparing meals for more than 20 years in enclosed cooking areas with elevated PM concentrations displayed lower FEV1/FVC ratios, consistent with an enhanced risk of chronic obstructive pulmonary disease (COPD). Preliminary results also indicate that PEF decreased during cooking for 58% of the participants, and FEV1/FVC decreased during cooking for 67% of respondents.

2260 Simultaneous Exposure to Concentrated Ambient Particles and Acrolein Causes Cardiac Effects Mediated by Parasympathetic Modulation in Mice

N. Kurhanewicz[1], A. Ledbetter[2], L. Walsh[2], A. K. Farraj[2] and M. S. Hazari[2]. [1]*Toxicology, University of North Carolina, Chapel Hill, NC and* [2]*US EPA, Environmental Public Health Division, Research Triangle Park, NC.*

Numerous studies show that short-term air pollution exposure modulates heart rate variability (HRV). HRV is a marker of autonomic influence on the heart and represents homeostatic control mechanisms regulating cardiovascular function. Our previous studies show that a single exposure to air pollution increases arrhythmia in mice in a manner implicating autonomic imbalance. Thus, the goal of this study was to characterize the autonomic changes due to exposure as reflected by HRV. We hypothesized that pharmacological inhibition of the parasympathetic and sympathetic effects on the heart would block the cardiac response to air pollution. Conscious, unrestrained C57BL/6 mice were exposed to ~40 ug/m3 concentrated ambient particles (CAPs) and 0.3 ppm acrolein for 3 hours. Immediately prior to exposure separate cohorts were treated with either atropine (2 mg/kg) or atenolol (2 mg/kg) to block the parasympathetic or sympathetic influence on the heart, respectively, hexamethonium (80 mg/kg) to block both, or saline. Electrocardiogram (ECG) and HR were recorded continuously before, during and after exposure. Treatment with atropine and hexamethonium increased HR and decreased HRV whereas atenolol did the opposite. Exposure to CAPs+acrolein did not affect HR, however SDNN and RMSSD both increased when compared with filtered air exposure. Pretreatment with both atropine and hexamethonium blocked this response whereas atenolol had no effect. Thus inhibition of parasympathetic influence blocked the HRV response (and arrhythmia) to air pollution exposure. These data demonstrate that even low level exposures to air pollution, which may not cause observable symptoms, can disrupt normal autonomic balance in healthy young animals and that these changes are characterized by parasympathetic dominance.(This abstract does not reflect US EPA policy)

2261 Serum-Borne Vascular Toxicity Following Inhalation of Mixed Engine Emissions and Treated Particles

J. Liu[1], J. Brower[1], M. Doyle-Eisele[1], G. Herbert[2], L. Heine[2], B. Sanchez[2], K. Zychowski[2], L. Topper[2], J. McDonald[1] and M. Campen[2]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM and* [2]*Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

Inhalation of vehicular emissions can induce inflammatory responses in the systemic vasculature, which may cause or exacerbate complex chronic vascular diseases such as atherosclerosis. We examined the changes of circulating bioactivity related to endothelial cell (EC) inflammatory responses and wound healing following inhalation of combustion-source mixtures or various components of mixed gasoline and diesel vehicle emissions (MVE) generated in the laboratory by combining gasoline and diesel engine exhausts. Male ApoE-/- mice were exposed to one of five atmospheres (50 days, 6 h/day): filtered air (control group), MVE100 (particulate matter concentration 100 µg/m3), MVE300 (PM concentration 300 µg/m3), MVE PM without gas phase components (MVE-gas), and MVE PM without semi-volatile organic compounds (SVOCs) (MVE-SVOC). Serum obtained from mice after the exposure was used to treat primary murine ECs at 5% vol/vol in media for 4h to examine inflammatory gene expression. Aortas obtained from the same mice were also examined for gene expression. Elevated vascular cell adhesion molecule 1 (VCAM-1) gene expression was found in ECs treated with HWS serum when compared to the control group, but not in the aortas where we found increased expression of matrix metallopeptidase 9 (MMP-9) and chemokine (C-C motif) ligand 2 (CCL2) in most exposed groups. Serum samples were also tested on ECs for an Electric Cell-Substrate Impedance Assay (ECIS) to assess its effect on endothelial barrier integrity and on cellular regrowth. Small impairments in regrowth after a wounding protocol were noted in cells incubated with serum from the MVE100 group. These findings are a first attempt to pair *ex vivo* serum bioactivity assays with *in vivo* outcomes. While *in vitro* bioactivity of the serum was noted, it was modest compared to *in vivo* gene expression, suggesting some accumulation of pathology that occurs to serum-exposed cells long-term.

2262 Serum from Diesel Exhaust-Exposed Rats with Cardiac Dysfunction Alters Aortic Endothelial Cell Function *In Vitro*: Circulating Mediators as Causative Factors?

A. K. Farraj[3], J. Shanahan[2], L. C. Thompson[3], C. M. Perez[1], N. Haykal-Coates[3], C. King[3], M. S. Hazari[3] and J. Brown[2]. [1]*Meredith College, Raleigh, NC;* [2]*Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO and* [3]*Environmental Public Health Division, US EPA, Research Triangle Park, NC.*

Although circulating inflammatory mediators are strongly associated with adverse cardiovascular outcomes triggered by inhaled air pollution, direct cause-effect linkage has not been established. Given that endothelial toxicity often precedes and precipitates cardiac dysfunction, the purpose of this study was to examine the plausibility of serum bound factors as drivers of both endothelial toxicity and cardiac effects. We hypothesized that serum taken from diesel exhaust (DE)-exposed rats that later develop cardiac dysfunction would alter aortic endothelial cell function *in vitro*. To assess cardiac function *in vivo*, left ventricular pressure assessments were conducted in spontaneously hypertensive rats one day after a single 4 hour whole body exposure to 150 or 500 µg/m3 DE or filtered air. Rat aortic endothelial cells (RAEC) were then exposed to diluted serum collected 1 hour after exposure from a separate cohort of similarly exposed rats for measures of VCAM-1, cell viability, nitric oxide synthase levels, and mRNA expression of key mediators of inflammation. Exposure of rats to either 150 or 500 µg/m3 DE decreased end systolic pressure and left ventricular pressure, consistent with a decrease in mean arterial pressure. Treatment of naïve RAECs to serum from rats exposed to 500 µg/m3 DE caused an increase in the cell surface marker VCAM-1 and a decrease in the expression of ALOX15 mRNA relative to cells treated with serum from rats exposed to filtered air. Serum from rats exposed to either 150 or 500 µg/m3 DE caused a decrease in cell viability and nitric oxide synthase production in RAECs relative to cells treated with serum from rats exposed to filtered air. Proteomic assessments of rat sera are currently being conducted to identify circulating mediators with altered expression after DE exposure. These results suggest that increases in circulating inflammatory mediators may contribute to the cardiac pathophysiology caused by exposure to air pollution (This abstract does not reflect US EPA policy; supported in part by NIH R01 ES019311 and K99 ES024392).

2263 Co-Exposure to Inhaled Ambient Particulate Matter and Acrolein Alters Myocardial Synchrony in Mice: Evidence for TRPA1 Involvement

L. C. Thompson, L. Walsh, N. Haykal-Coates, A. D. Ledbetter, D. Davies, M. Higuchi, W. E. Cascio, M. S. Hazari and A. K. Farraj. *Environmental Public Health Division, US EPA, Research Triangle Park, NC.*

Air pollution is a complex mixture of constituents, often including particulates and aldehydes. As such, attributing health effects to air pollutants in a given ambient air shed can be difficult when pollutants are studied in isolation. The purpose of this study was to examine the cardiovascular effects of repeated, intermittent co-exposure to concentrated ambient particulate matter (CAPs) and acrolein. We hypothesized that co-exposure would cause greater deficits in myocardial synchrony and performance than exposure to either pollutant alone, and that such effects would be mediated by activation of the transient receptor potential cation channel (TRPA1), a mechanism we previously linked to diesel exhaust-induced increases in cardiac arrhythmogenesis. Female B6129 (WT) mice and Trpa1-/- mice (n=6) were exposed to filtered air (FA), CAPs (~45 µg/m3 of PM2.5 approx. 150 nm diameter), acrolein (0.42 ppm) or CAPs+acrolein for 3 hours a day, 2 days a week, for 4 weeks. Cardiac dimensions, strain data, and mechanical function were investigated with echocardiography (at 40 MHz) before the onset of exposures, 1 day after the first exposure, and 1 day after the final exposure. Preliminary results from after the final exposure indicate that CAPs+acrolein increased myocardial strain delay (indicator of cardiac dyssynchrony) in WT mice by ~5-fold when compared to delay in WT mice exposed to FA, CAPs, or acrolein alone; and when compared to delay in Trpa1-/- mice exposed to CAPs+acrolein. There were no significant differences in strain delay between WT mice exposed to FA, CAPs, or acrolein alone and the corresponding exposure groups in Trpa1-/- mice. Indicators of myocardial performance were not different between groups at the final time-point. These results demonstrate 1) greater than additive cardiac effects of CAPs and acrolein co-exposure and 2) TRPA1 involvement in the loss of cardiac synchrony induced by CAPs and acrolein co-exposure. (This abstract does not reflect US EPA policy).

2264 Effect of Exposure to Different Size Fractions of Concentrated Ambient Particles on Blood Pressure

M. N. Saiduddin, A. Keebaugh and M. Kleinman. *Department of Medicine, University of California Irvine, Irvine, CA.*

Particulate matter is a heterogeneous mixture of particles that are suspended in the atmosphere. These particles have diverse chemical compositions and sizes, and their sources can range from urban emissions to wildfires. Ambient air pollution has been investigated as a possible factor in increased blood pressure. Of the types of particulate matter, ultrafine particles (diameter ≤ 0.18 µm) are thought to be the most toxic. They have the greatest surface area-to-volume ratio, and are therefore more biologically available. This study seeks to compare acute and chronic changes in blood pressure of mice exposed to different sized fractions of concentrated ambient particles (CAPs). BALB/c mice were exposed to ultrafine, fine (diameter ≤ 2.5 µm), coarse (diameter ≤ 10 µm) CAPs, or filtered air five hours a day, four days a week, for nine months. Blood pressure for each mouse was measured immediately after exposure once every three months. At each three-month interval, the ultrafine group was seen to have the highest post-exposure blood pressure. However, the response was only significant at the three-month time point, indicating that the effect may become blunted with chronic exposures. For air, coarse, and fine groups, blood pressure values did not differ significantly from baseline. These results indicate that the ultrafine particles have the greatest impact on acute blood pressure change in mice (similar to what has been seen in human epidemiology studies). With this model, further investigation can be performed into the mechanism responsible for this blood pressure increase.

2265 Electrocardiogram Changes in ApoE-/- Mice Following Exposure to the Semi-Volatile Fraction of Ultrafine Concentrated Ambient Particulate Matter

D. A. Herman, A. Keebaugh and M. T. Kleinman. *Environmental Health Sciences, University of California, Irvine, Irvine, CA.*

Air pollution has been associated with increased incidence of cardiovascular disease leading to increased daily mortality and hospital admissions. Urban airborne ultrafine particulate matter (UFPM) contains semi-volatile organics (SVOCs) which have a high proportion of redox active compounds that can be bound to particles or can partition to the vapor phase after emission where they are responsible for some of the observed exacerbation of PM-associated adverse cardiovascular health effects. Therefore, we showed that removal of SVOCs from an aerosol decreased the ability of UFPM to cause oxidative damage and consequently increase cardiac abnormalities measured by changes in electrocardiogram (ECG) waveform morphology. However, when the organics were removed some of the particle mass was reduced. Therefore, we tested whether the reduction of toxicity was solely due to the removal of organics or if the reduced particle mass was important as well. Groups of genetically altered ApoE -/- mice (prone to developing atherosclerosis) were exposed to either purified air, quasi-ultrafine concentrated ambient particles (CAPs; under 0.18µm), denuded CAPs (deCAPs) from which SVOCs were removed from the particle core using a thermal denuder, or to CAPs diluted (DilCAPs) to the same concentration as DeCAPs. Mice were exposed 5 hours/day, 4 days/week for 8 weeks at the University of California, Irvine. Implanted cardiac transducers monitored ECGs from the mice which were then analyzed to detect changes from the baseline in the QT segment, J-point, and S-T segment. Mice exposed to any form of the UFPM atmospheres showed an increase in abnormal waveform morphologies over the control group. Furthermore, Mice exposed to the entire fraction of the UFPM showed increased abnormalities over mice exposed to UFPM lacking the SVOC fraction. There is some evidence of a relationship between the QT segment changes and both UFPM concentration and total measured HRV. These results elucidate that a dose-dependent relationship is not required for these morphological changes to occur at equal proportions. The measured morphological changes in these ECG waveforms are common metrics of myocardial dysfunction in humans that may lead to life-threatening cardiac events. Further investigation into the observed changes need be performed to explicate the mechanisms of the changes resulting from these PM exposures.

2266 Characterization and Mutagenicity of Smoke from Smoldering and Flaming Combustion of Peat and Red Oak Biomass Fuels

Y. H. Kim[2], S. Warren[2], Q. T. Krantz[2], C. King[2], R. Jaskot[2], M. Hays[2], M. Landis[2], M. Higuchi[2], D. DeMarini[2] and M. I. Gilmour[2]. [1]*NERL, US EPA, Research Triangle Park, NC and* [2]*NHEERL, US EPA, Research Triangle Park, NC;* [3]*NRMRL, US EPA, Research Triangle Park, NC.*

Although wildfire smoke is known to cause adverse health effects, less is known about the relative effects of wildfire smoke from different fuel types or combustion conditions. In this study, we describe a novel in-tandem application of controlled combustion and cryo-trapping techniques that utilize an automated tube furnace system to simulate wildfire combustion and facilitates the efficient collection of the resulting smoke emissions. The furnace sustained stable flaming and smoldering biomass (red oak, peat) burning conditions consistently for ~60 min. The multi-stage cryo-trapping system collected up to 90% of the biomass combustion emissions at -70°C. Condensates were extracted and assessed for mutagenicity in Salmonella strain TA98+/-S9. Carbon dioxide, carbon monoxide (CO), and particulate matter (PM) concentrations were monitored continuously during the combustion process and used to calculate the modified combustion efficiency (MCE) and emission factors (EFs). We found that the MCE during smoldering conditions was 71% and 74% and during flaming conditions was 96% and 99% for peat and red oak, respectively. Red oak smoldering EFs for CO and PM were 209 g/kg and 147 g/kg, whereas flaming EFs were 16 g/kg and 0.6 g/kg, respectively. Peat smoldering EFs for CO and PM were 301 g/kg and 59 g/kg, respectively, whereas peat flaming EFs were 47 g/kg and 3 g/kg. The ranking of the fuels based on mutagenicity-emission factor in TA98+S9 (revertants x 105/kg fuel) was red oak smoldering (137) > peat smoldering (95) > peat flaming (19) > red oak flaming (2). Our results demonstrate that type of fuel and combustion conditions have dramatic differences in emission characteristics, including pollutant and mutagenicity EFs. The successful application of the novel combustion and sample-collection system presented demonstrates its efficacy for use in future assessments of simulated wildfire emissions. [This abstract does not represent official US EPA policy.]

2267 Development of a Method to Estimate *De Minimis* Levels for Metals in Meat and Poultry

J. G. Muniz Ortiz[1], R. Duverna[1], A. Domesle[2] and K. Dearfield[1]. [1]*USDA, Food Safety and Inspection Service, Washington, DC and* [2]*USDA, Office of Pest Management Policy, Washington, DC.*

The Food Safety and Inspection Service (FSIS) conducted a dietary exposure assessment to determine if consumption levels from FSIS-regulated products might pose a potential adverse health outcome. The assessment used data from the National Residue Program (NRP) and examined the identified mean and maximum concentrations of each metal from the muscle tissues of each of the animal classes sampled (bovine, porcine, chicken, and turkey) from 2012 to 2015. For many years, FSIS has monitored meat, poultry and egg products for chemical residues through the NRP. Along with lead and cadmium which have been quantified since 2003, FSIS began testing for other trace elements, including cobalt, manganese, molybdenum, and selenium. These metals are essential dietary micro-nutrients; however, excessive dietary intake of these metals could pose a public health risk. The results from this assessment showed that levels of these metals found in FSIS-regulated commodities and consumed by the population are below 100% of the respective Health-Based Guideline Value (HBGV) of each metal. Based on this assessment, we developed a method to estimate the concentration of each metal that would result in consumption of 100% of the HBGV from each animal class. These estimates could be considered a level of no public health concern, or *de minimis*, if consumption of the mean level of the metal via FSIS-regulated product occurred chronically. A comparison of the concentrations identified via NRP sampling and the estimated *de minimis* levels were below a level that would pose an adverse health outcome. We will be incorporating consumption rates of each metal harbored in non-FSIS-regulated food product to allocate the *de minimis* level to FSIS-regulated product accordingly. These approaches will allow FSIS to compare the concentrations of each metal harbored in each animal class to a level that would not pose a health risk to consumers.

2268 Clinical Safety Evaluation of the Novel Probiotic *Bacillus subtilis* CU1

M. Lefevre[2], P. Jüsten[2] and A. R. Lobach[1]. [1]*Intertek Scientific & Regulatory Consultancy, Mississauga, ON, Canada and* [2]*Lesaffre Human Care, Lesaffre Group, Marcq en Baroeul, France.*

Background: Current probiotic preparations on the market are composed of a number of different live bacterial species, that when consumed in sufficient quantities, confer a health benefit to the host. *Bacillus subtilis* has gained recent interest as a commercial probiotic due to the fact that as a spore-forming bacterium it is thermo-resistant and stable over a wide pH range. Here we describe the strain *Bacillus subtilis* CU1, a novel probiotic, and present clinical data demonstrating its safety for human consumption. Methods: A randomized, double-blind, placebo-controlled, parallel-arm study was conducted in healthy free-living elderly subjects, approved by the West IV Ethics Committee for Human Research and the French Health Products Safety Agency. Subjects consumed one capsule per day containing either *Bacillus subtilis* CU1 (2 x 109 spores/capsule, n=50) or placebo (n=50) for 10 days, followed by an 18-day washout period. The consumption/washout cycle was repeated 3 times for a total of 16 weeks. The occurrence of adverse events was documented by the study investigators in the case report forms for each subject. Blood was collected at baseline and study endpoint (week 16) for complete blood counts and clinical chemistry evaluation of liver and kidney markers. Hemodynamic parameters, including arterial pressure and heart rate, were evaluated at baseline, week 8, and week 16. Results: The total number of adverse events in the group consuming *Bacillus subtilis* CU1 was not statistically different than the placebo (P=0.8369, chi-square test) and overall, probiotic consumption was not associated with any undesirable physiological effects. Markers of liver (ALT, AST, GGT) and kidney (creatinine, urea) function, complete blood counts, and hemodynamic parameters were unaffected by *Bacillus subtilis* CU1. Conclusion: Overall, the consumption of *Bacillus subtilis* CU1 (2 x 109 spores/day) for a total of 40 days was found to be safe and well-tolerated by all subjects and supports the safe use of *Bacillus subtilis* CU1 as a probiotic.

2269 Dietary Questionnaire of Maize Consumption and Aflatoxin Content of Corn-Based Food Samples from Street Markets in the Metropolitan Area of Monterrey, Nuevo Leon, Mexico

R. S. Treviño-Espinosa[2], A. J. Ruiz-Uribe[2], R. M. Sanchez-Casas[2], P. Gonzalez-Barranco[1], M. Chavez-Bautista[2], S. E. Elmore[3], T. D. Phillips[3] and A. G. Marroquin-Cardona[2]. [1]*Faculty of Chemistry, Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, NL, Mexico;* [2]*Toxicology, Faculty of Veterinary Medicine, Universidad Autonoma de Nuevo Leon, Gral. Escobedo, NL, Mexico and* [3]*Veterinary Integrative Biosciences, Texas A&M University, College Station, TX.*

Maize is the most important staple food in Mexican culture and aflatoxins are common contaminants that can be present in corn-based products. In order to know the preferences of consumption of maize and maize products, a questionnaire was applied to a population in northern Mexico. Additionally, the levels of total aflatoxins (AFs) were determined in food samples of the most consumed products by this population. Participant recruitment in the metropolitan area of Monterrey (9 cities), was based on age (between 18 and 75 years old) and positive response of consumption of corn products from street markets. Proper bioethics permits were obtained. A total of 108 questionnaires were applied to 55 women and 53 men, respectively. Results indicated that the most consumed foods were tortillas (100%); Mexican food (99.07%); corn products (95.37%) and canned corn (80.56 %). Considering these results, we collected 10 samples of the most popular foods from street markets from 6 cities. Total aflatoxins were determined with fluorometer (VICAM®) using immunoaffinity columns (AflaTest®). Most of the samples had AFs levels within the permitted action limits (< 12 µg/kg) according to Official Mexican Standards (NOM-247-SSA1-2008). Average AFs levels were 4.98 µg/kg in sampled foods, with 21 µg/kg as the highest, and 1.2 µg/kg as the lowest concentrations found, respectively. Ongoing work is focusing on analyzing more samples to reach a total of 10 samples from each city. Funding: CONACYT-TAMU 2014 Project 035

2270 Development of a Noncancer Toxicity Criterion for Triethylene Glycol (TEG) Using Developmental Toxicity Data as the Most Sensitive Endpoint

L. A. Beyer[1], M. L. Hixon[1], D. B. Mayfield[2], M. K. Peterson[2] and E. M. Dubé[1]. [1]*Gradient, Cambridge, MA and* [2]*Gradient, Seattle, WA.*

Mislabeling of shipping containers by a supplier resulted in the inadvertent contamination of coffee with triethylene glycol (TEG). We analyzed coffee sample data and toxicity information to determine whether the concentrations of TEG detected in the contaminated coffee could result in adverse health effects. We evaluated potential TEG doses based on average (1.5 cups per day) and high-end (4 cups per day) coffee consumption. Next, based on the scientific literature, we determined that TEG is not carcinogenic, and we developed a noncancer toxicity criterion since none was available. In accordance with United States Environmental Protection Agency (US EPA) guidance, we calculated an oral reference dose (RfD) of 0.39 mg/kg-d. This RfD was based on a no observed adverse effect level (NOAEL) for the critical endpoint (fetotoxicity) in the most sensitive species (the mouse) and standard uncertainty/safety factors recommended by US EPA. We compared potential TEG exposure from drinking the contaminated coffee to the RfD by calculating a hazard index (HI) for subchronic exposure such as that potentially experienced by consumers of the contaminated coffee. Exposure was evaluated using the mean TEG concentration (0.44 mg/mL) and the 95% upper confidence limit on the mean (UCLM) concentration of TEG (0.49 mg/mL) in brewed coffee. Finally, we compared exposure to the RfD by calculating a hazard index (HI) in which the hypothetical estimated exposure (numerator) was compared to the toxicity criterion (denominator). An HI is the ratio of the potential exposure to the substance and the level at which no adverse effects are expected. If the HI is equal to or less than 1, then no adverse health effects are expected as a result of exposure. Using the 95% UCLM concentration resulted in an HI of 0.33 for average coffee consumption and an HI of 0.97 for high-end coffee consumption. Based on our analysis, reported levels of TEG as a contaminant in coffee posed no risk of adverse health effects to consumers, based on either average or high-end coffee consumption data. Our approach can be used by others in the food industry to evaluate possible toxic effects from contaminants and to help determine whether a product recall is needed.

2271 Aflatoxins Control in Milk and Dairy Feeds in the Mexican Highland Plateau

A. G. Valdivia[2], E. J. Rangel-Muñoz[2], C. A. Miranda-Castañeda[2], C. R. Cruz-Vazquez[1], T. Quezada-Tristan[2], R. Ortiz-Martinez[2] and L. E. Medina-Esparza[1]. [1]*Instituto Tecnologico El Llano, Aguascalientes, Mexico and* [2]*Departamento de Clinica Veterinaria, Universidad Autonoma de Aguascalientes, Aguascalientes, Mexico.* Sponsor: G. Pallas-Guzman.

The aflatoxins (AF) are toxic and carcinogenic compounds produced by fungi of *Aspergillus* genus. The aim of this study was to evaluate the contamination in feed and milk produced on dairy farms by toxigenic fungi and AF. During 29 months (2012–2014), samples of corn silage, concentrates, total mixed ration (RTM) and raw milk were obtained in two dairy farms (2,842 cows) in The Mexican Highland Plateau; the RTM was added with several commercial mycotoxin binder products. The fungi were isolated and identified by optical and scanning electron microscopy; their toxicogenic capacity was also tested (TLC). AF were measured in feed and raw milk (ELISA and HPLC). Although 99% of the samples were AF contaminated, only 21% presented *Aspergillus spp*. isolation and 17% showed aflatoxicogenic capacity. A significant difference (P<0.05) between the AF concentration in feedstuffs and the AF in RTM (33.3±2.5 vs 12.4±1.0 AF µg/kg of feed), which representing a 62.8% of average efficiency. During the study cows were exposed to AF (627±0.43 µg/cow/d), but a marginal amount (0.93%) of original AF ingested was eliminated in milk as aflatoxin M1. These results suggest that the addition of mycotoxin binder has the potential to reduce the transference rate of AF to raw milk in field conditions. This is relevant to human and animal health in order to reduce the risk of exposure and damage.

2272 Longitudinal Aflatoxin Exposure in Pregnant Women in East Kenya

L. Tang[2], K. S. Xue[2], M.-s. Kang[2], J. Lerory[1], V. Hoffmann[1], K. Jones[1] and J.-S. Wang[2]. *[1]International Food Policy Research Institute, Washington, DC and [2]University of Georgia, Athens, GA.* Sponsor: J.-S. Wang.

Aflatoxins (AFs) are health threaten mycotoxins which have long been recognized as hazardous contaminants of food, especially in peanuts and corn. Aflatoxin B1 (AFB1) is a potent hepatotoxic and genotoxic agent, and has been categorized as a known human carcinogen (Group I) by IARC. Acute exposure to high levels of AFB1 via the diet caused disease (aflatoxicosis) and death in humans including outbreaks in east Kenya in 2004–2005. Chronic exposure to low levels of AFs is one of the major risk factors of human hepatocellular carcinoma (HCC) in several regions of Africa and Southeast Asia. Importantly, AFB1 has also been shown to be an anti-nutritional agent that reduced vitamins and proteins in animals and humans, and is a potent immunotoxic agent in animals and humans, which may aggravate the infectious diseases in developing world. Impairment in children's growth and development as a consequence of anti-nutritional and immune toxic effects has recently been a great public health concern. The objective of this study is to study AFB1 exposure in pregnant women in Eastern Kenya in order to assess the potential adverse effect on various health outcomes. Blood samples were collected from pregnant women who reside in 65 villages in Meru in the Eastern province of Kenya. Serum was separated, aliquotted into microcentrifuge tubes, and shipped via air to US laboratory for analysis of AFB1-lysine adduct. AFB1-Lysine adduct was detectable in all analyzed 2,026 samples collected from 6 different waves in two years. The median level of the adduct (4.24 pg/mg albumin) in wave 4 samples was the lowest and the median level of 45.08 pg/mg albumin in wave 5 sample was the highest. According to the median levels, the order among 6 waves was 45.08 (wave 5) > 36.01 (wave 6) > 8.82 (wave 3) > 7.96 (wave 2) > 5.95 (wave 1) > 4.24 (wave 4). According to mean level, the order among 6 waves was 77.99 (wave 6) > 69.75 (wave 5) > 28.01 (wave 2) > 24.36 (wave 3) > 18.73 (wave 1) > 9.46 (wave 4). The frequency distribution of the adduct levels was significant different among 6 waves ($p<0.001$), which indicated great temporal and individual variations in AFB1 exposure among study populations.

2273 Using Reported Adverse Events to Identify Potential Health Risks and Individual Susceptibility Associated with Energy Drink Consumption

D. A. Drechsel[1], A. Banducci[1], M. Nelson[2], R. Novick[2] and B. Tvermoes[1]. *[1]Cardno ChemRisk, Boulder, CO and [2]Cardno ChemRisk, San Francisco, CA.*

The popularity of energy drinks has grown substantially in the US and worldwide over the past three decades. Energy drinks typically contain caffeine and other ingredients such as taurine, guarana, and B vitamins with purported claims of reducing fatigue, enhancing mental focus, and increasing physical performance. Recent reports have suggested that an increasing number of adverse health events related to energy drink consumption have occurred since the introduction of these products in the late 1990s. In addition, concerns have been expressed regarding the caffeine content of energy drinks and possible interactions between ingredients. This study utilized published case reports and data available from the FDA's Adverse Event Reporting System to compile information on the number and type of adverse health events associated with the ingestion of energy drinks. A search of the available literature identified 52 case reports that provided detailed medical information. The majority of cases involved individuals 25 years of age or younger. Patients' clinical symptoms generally fell into three main categories: cardiovascular (27 cases), neurological (9 cases), and psychiatric (9 cases). Cases with symptoms related to hepatic (4 cases) and renal (2 cases) systems, along with one case of elevated vitamin B were also identified. It was generally not possible to conclude that a specific ingredient was more frequently associated with adverse symptoms due to the lack of information regarding the type of energy drink or quantity consumed. However, in cases where approximate intake was known, seizures were associated with doses of > 300 mg caffeine in patients with prior history of such neurological events. We also reviewed over 300 reports of adverse health events associated with energy drink consumption reported to the FDA from 1997 to 2014. Similar to the case reports, cardiovascular- and neurologic-related symptoms were most commonly reported. The majority of reports involving serious adverse events or life-threatening symptoms were associated with underlying conditions such as a weakened cardiovascular system. Understanding how these preexisting conditions may increase one's susceptibility to caffeine and other common ingredients in energy drinks presents an interesting avenue of research to identify the potential health risks in vulnerable populations.

2274 Current Status of Aflatoxin B1 Contamination in Grains and Peanuts Grown in the State of Georgia, USA

A. G. Seawright, L. Tang and J. S. Wang. *Environmental Health Science, University of Georgia, Athens, GA.*

Aflatoxins (AFs) are the most common naturally occurring mycotoxins produced mainly by *Aspergillus flavus* and *A. paraciticus*. AFB1 is carcinogenic, immunosuppressive, mutagenic, potentiates other diseases, and is harmful to both animals and humans that frequently consume AFB1-contaminated foods. The State of Georgia's climate during grain and peanut growing and harvesting seasons provides ideal conditions for growth of the toxigenic fungi that produce AFs. The purpose of this study is to investigate the current state of AF contamination in Georgia grown grains and peanuts because no such information is available over two decades. Approximately 200 grain samples and 150 peanut samples were collected from various sources, including farmers, USDA and FSIS stocks, and local markets. Different extraction and analysis methods were adopted and compared for their capability in detecting AFs in peanuts and grains. Analytical protocols were developed and validated via using spiked samples and selection of ratios of solvent extraction for different matrixes. Samples were first analyzed using ELISA method, and then confirmed by ultra-high-performance liquid chromatography (UHPLC) method. Ongoing analysis shows that 92% of grains and peanuts have detectable levels of AFB1 with a range of 0–18 ppb. However, all detected levels are below the regulatory limit of 20 ppb set by the US FDA. This data will provide useful information for state government authorities and regulatory agencies to understand the current status and what steps should be taken to decrease AF levels in commodities and food products, especially under the trend of climate changes.

2275 Assessing Hazard Potential of Lead Exposure in Children or Adults: Case Studies of Two Food Products

B. Flannery, D. Smegal and J. Spungen. *Division of Risk and Decision Analysis, US FDA, Center for Food Safety and Applied Nutrition, College Park, MD.*

Part of FDA's mission is to protect public health by assuring safety of the United States' food supply, which includes monitoring for metals and other contaminants in food. A contaminant of particular interest is lead (Pb) due to its environmental abundance and its potential to cause adverse neurodevelopment effects in children. Here, our goal was to assess the lead hazard potential of two existing food products to support regulatory decision making. Product 1 contained 416 ppb Pb and was a food assessed only in children. Product 2 contained 48.8 ppm Pb and was a food only consumed by adults. First, we compared lead exposure from individual products to the FDA Provisional Total Tolerable Daily Intake (PTTDI) for lead of 6 ug/day for children and 75 ug/day for adults. Then, we estimated blood lead levels (BLL) likely to result from consumption of these individual products using FDA methodology. Finally, we used EPA's Integrated Exposure Uptake Biokinetic (IEUBK) and EPA's Adult Lead Methodology (ALM) models to confirm our BLL estimates. Exposure estimates showed daily lead intake from each product exceeded the FDA's PTTDI for the respective populations, and thus would be considered to be adulterated and injurious to health. FDA methodology estimated BLLs from products 1 and 2 to be 7 ug/dL for children, and 22.5 ug/dL for adults, respectively, which exceeded the CDC's BLL guideline of ≤ 5 ug/dL for children and ≤ 10 ug/dL for non-pregnant adults. IEUBK modeling estimated consumption of product 1 would result in a BLL of 3.9 ug/dL, while ALM modeling estimated consumption of product 2 would result in a BLL of 26 ug/dL. Slight differences in BLLs in FDA methodology and the models are likely due to differences in model assumptions including absorption. Taken together, this information demonstrates the use of modeling tools to assist in making regulatory recommendations.

2276 *Fusarium* Mycotoxins Determination and Their Dietary Intake through Wheat and Flour Wheat by the Romanian Population

C. Juan[3], O. Stanciu[2], F. Loghin[2], D. Miere[2], M.-J. Ruiz[3] and J. Mañes[3]. [1]*Dep. of Bromatology, Hygiene and Nutrition, Iuliu Haţieganu Uni. of Medicine and Pharmacy, Cluj-Napoca, Romania;* [2]*Dep. of Toxicology, Iuliu Haţieganu Uni. of Medicine and Pharmacy, Cluj-Napoca, Romania and* [3]*Laboratory of Food Chemistry and Toxicology, University of Valencia, Valencia, Spain.* Sponsor: G. Font.

Food safety is a priority of the European Commission, which has established maximum or recommended levels for some mycotoxins. *Fusarium* mycotoxins in unprocessed wheat and wheat flour, maximum levels (ML) have been set for deoxynivalenol (1750 and 750 µg/kg, respectively) and zearalenone (100 and 75 µg/kg, respectively), and indicative levels have been set for the sum of HT-2 and T-2 toxins (100 and 50 µg/kg, respectively) (2006/1881/CE; 2013/165/UE). In this study, 31 Romanian wheat samples and 35 flour samples collected in 2014 were analyzed the presence of deoxynivalenol (DON), 3 and 15-acetyldeoxynivalenol (15-AcDON and 3-AcDON), nivalenol (NIV), diacetoxyscirpenol (DAS), neosolaniol (NEO), HT-2 and T-2 toxins, beauvericin (BEA), enniatins (ENA, ENA1, ENB, ENB1), and zearalenone (ZEA). Analysis was performed using liquid chromatography coupled to tandem mass spectrometry. Limits of quantification ranged between 1.6 and 313 µg/kg. For the 66 analyzed samples, analytical results showed that the investigated samples contained DON, ZEA, NEO and ENs. DON was detected in the 26% and 3% of wheat and flour analyzed samples, respectively, while ZEA was detected in the 13% and 6%, respectively. NEO occurred in one flour sample. For enniatins, the highest incidence was for ENB, the occurrence being 61% for wheat, and 80% for flour samples. Regarding the values detected, one and four samples of wheat were above ML of DON and ZEA, respectively. Knowing the mean values for the detected mycotoxins and the wheat and products supply quantity for Romanian population according to FAOSTAT (365 g/capita/day), the dietary intake for each mycotoxin was estimated: for DON 0.57 µg/kg bw/day, for ZEA 0.26 µg/kg bw/day, for ENB 0.27 µg/kg bw/day, for NEO 3.5 pg/kg bw/day. Due to DON, ZEA and ENs contamination, further research is necessary to confirm their presence in food and also to estimate the dietary intake for Romanian population. ACK: Projects, Spanish Ministry AGL2013-43194-P, AGL2014-52648-REDT and European Social Fund POSDRU/159/1.5/S/136893.

2277 Fluoride: Friend or Foe?

B. Kelman[2], R. Evoy[1,2], C. Chan[2] and N. Moore[2]. [1]*Columbia University, New York, NY and* [2]*Veritox, Inc., Redmond, WA.*

Early in the 20th century tooth decay was widespread. Epidemiology studies revealed although high levels of fluoride (F) in community water systems (CWS) were associated with dental fluorosis, low levels were protective against caries. Studies by HT Dean indicated about 1.0 mg/L F in CWS could reduce caries in children while minimizing dental fluorosis and, in 1962, the US Public Health Service (PHS) recommended fluoridation of CWS to protect against caries. Fluoridation critics have long questioned F's efficacy, its potential for dental fluorosis, and its potential to cause major adverse human health effects. In 2015, the PHS cited increased prevalence of child dental fluorosis to reduce optimal F in CWS to 0.7 mg/L. Many factors have changed since Dean's initial studies with the most noteworthy including expansion of exposure sources beyond CWS to include bottled water, dental products, and dietary supplements. We performed a risk assessment comparing F exposures early in the 20th century to those early in the 21st century to clarify contributions driving the increased prevalence of dental fluorosis and estimate current exposure levels associated with 0.7 and 1.0 mg/L in CWS. We confirmed today's children <1, 1-3, 4-6, 7-10, and 11-14 years of age have larger F exposure than those during the time of Dean (1942). Specifically, average F exposure during the time of Dean were estimated using drinking water (DW) and diet studies; similar DW/beverage and diet studies were used to calculate current intakes; additional sources of exposure were included to estimate total current intakes; and total current intakes were converted to corresponding DW levels during the time of Dean. For children 1-3 years old, 1.0 mg/L CWS resulted in exposures of 0.65 mg/d (during the time of Dean) and 1.3 mg/d (currently, with additional F sources). The current exposure of 0.65 mg/d correlated with a 2.0 mg/L CWS during the time of Dean (when the fluorosis rate was 55%). Current exposure paradigms associated with consumption of CWS with 0.7 mg/L indicated 1-3 year olds have an average exposure of 1.2 mg/d (with additional F sources); this correlated with a 1.8 mg/L CWS during Dean's studies (and well above Dean's optimal 1.0 mg/L). Our analysis suggests PHS' reduction in optimal F levels is not sufficient to reduce fluorosis levels due to the contribution of F from non-water sources.

2278 Evaluation of Metals in Different Species of Edible Mushrooms

M.-C. Rubio-Armendariz[1], A. Puteri[1], G. Luis-Gonzalez[1], A. Gutierrez-Fernandez[1], D. Glez-Weller[1], A. Hardisson[1] and A. R. Anadon[2]. [1]*Toxicology, Universidad de La Laguna, La Laguna, Spain and* [2]*Toxicology and Pharmacology, Universidad Complutense de Madrid, Madrid, Spain.*

Western European countries are the biggest consumers of mushrooms in the world. Boletes, Morels, Chantarelles and Truffles are the most popular species in western countries, and the Matsutake is the most popular in Japan. Objectives: 1) to quantify the concentrations of metals (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sr, V, Zn) in 35 samples of 3 species of edible mushrooms (*Agaricus bisporus, Lendidula* edodes and *Pleurotus ostreatus*), 2) to assess the intake of essential metals from the mushrooms and their contribution to the Recommended Dietary Intakes (RDIs), and 3) to evaluate the intake of toxic metals and their contribution to the corresponding Provisional Tolerable Weekly Intake (PTWI) and Tolerable Dairy Intake (TDI). Materials and methods: 35 samples of 3 species of edible mushrooms (*Agaricus bisporus, Lendidula edodes* and *Pleurotus ostreatus*) from different production origins (China, Korea and Spain) and purchased according to sale point (supermarket and shop) on the island of Tenerife (Spain) were analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Results: Range concentrations of metals in edible mushrooms were (mg/kg): Al (0.46-39.6), B (0.03-10.4), Ba (0.02-3.87), Ca (5.55-3345), Cd (0-0.41), Co (0.002-0.05), Cr (0.003-1.12), Cu (0.37-1554), Fe (1.71-106), K (224-5863), Li (0-14.9), Mg (19.6- 931), Mn (0.04-8.47), Mo (0.002-0.23), Na (27.3-13897), Ni (0.02-67.1), Pb (0.002-0.23), Sr (0.02-49.2), V (0.01-1.02), Zn (1.02-52.7). *Lendidula edodes* had the highest contents of metals while the lowest concentrations were found in *Pleurotus ostreatus*. Mushrooms of Spanish origin had the highest content (mg/kg) of B (1.38), Ca (332), Cu (31.6), Ni (1.41) and V (0.11). Conclusions: Edible mushrooms should be considered as a dietary source of metals. Mushroom consumption (100 g/adult/day) does not lead to a significant intake of toxic metals; therefore, there is no toxicological risk from daily consumption of mushrooms.

2279 Effect of Substitution and Stereochemistry on the Toxicity of Phenols and Organophosphorus Compounds

S. Mosley, T. B. Adams, S. Stice and A. Mattia. *US FDA, College Park, MD.* Sponsor: L. Rosenfeld.

The United States Food and Drug Administration's Office of Food Additive Safety is developing a program to prioritize the toxic potential of a wide variety of structurally-related food ingredients. The toxicity of substituted phenols and organophosphorus compounds in laboratory animals was analyzed based on stereochemistry, steric effects, and species-specific metabolic differences. The positions and sizes of the substituents and their effects on Phase I quinone formation and Phase II conjugation of phenols are correlated with toxicity in different animal models. The size of alkyl- and aryl-substituted phosphites is correlated with the potential for oxidation of the phosphorous atom and subsequent toxicity. The size of alkyl- and aryl-substituted phosphate esters is related to the ability to react with serine in acetylcholinesterase to inhibit this enzyme. These findings have been incorporated into a more robust tool for prioritizing the toxic potential of food ingredients compared to those in existing structure-based decision trees. The updated decision tree takes into account the current "state of the science" and provides regulatory bodies with a prioritization tool to improve the safety assessment of ingredients added to food.

2280 The Application of an Updated Cramer et al. Decision Tree to Food Ingredient Safety Assessment

S. Stice. *US FDA, College Park, MD.* Sponsor: S. Choudhuri.

The Cramer et al., Decision Tree (DT), published in 1978, provided a tool to prioritize orally ingested substances according to their toxic potentials. Based on the knowledge of chemical structure, metabolism, and toxicity, a sequence of yes/no questions were devised that leads to the assignment of a substance to one of three structural classes of toxic concern. Given the scientific knowledge accumulated since 1978, the DT sequence is long overdue for revision and expansion. More than 12,000 scientific studies were reviewed to determine the effects of species, strain, sex, and target organ on toxicity and metabolic fate. These studies provided no-observed-effect-levels (NOELs) for more than 1,800 substances that were then organized according to their toxicity and struc-

ture. This resulted in the ability to develop more refined DT questions leading to an increased number of classes of toxic concern. Substances in Class I are common components of food that are completely metabolized to CO2 and H2O; Class II includes mainly innocuous substances of low toxicity; Class III substances are of intermediate toxicity, many with species-specific differences that impact the assessment of human risk; Class IV substances are more toxic with structural features that suggest toxicity; Class V contains substances with a higher level of concern even at low levels of exposure; and finally, Class VI substances are highly reactive even at extremely low levels. These classes of toxic potential can be applied to a vast group of food ingredients to be used for prioritization and future evaluation.

PS 2281 An Exposure and Health Risk Assessment of Lead (Pb) in Chocolate

M. M. Abramson[1], A. D. Monnot[2] and W. V. Christian[1]. [1]*Cardno ChemRisk, Pittsburgh, PA and* [2]*Cardno ChemRisk, San Francisco, CA.*

Since 2002, the American Environmental Safety Institute (AESI) and other consumer groups have expressed concern about Pb levels in chocolate under California's Proposition 65. AESI reported that Pb was detected in 68% of tested chocolate products at concentrations ranging from 0.002 - 0.105 ppm. Pb levels up to 0.25 ppm in chocolate powders and 0.14 ppm in chocolate bars have also been reported in the literature. To assess whether these products pose a health risk to adults and children that regularly consume chocolate, we used the EPA's Adult Lead Methodology (ALM) and Integrated Exposure Uptake Biokinetic (IEUBK) models to predict the blood Pb levels (BLLs) of adults and children (aged 1-7) under varying exposure scenarios. Estimated background Pb exposures were combined with several ingestion scenarios reflecting "average" and "frequent" consumption of chocolate. Modeled BLLs were then compared to various health-based standards to assess the risk to consumers. We found that in all cases background Pb exposure was the primary contributor to estimated BLLs in children and adults, and Pb exposure from chocolate did not significantly increase estimated BLLs. Even assuming the highest Pb concentration reported in the literature for chocolate bars, 0.14 ppm, children and adults with average background Pb exposures would need to consume 2-9 and 10-20 chocolate bars (41 g each) per day to raise their BLL to the CDC and EPA BLLs of interest, 5 µg/dL and 10 µg/dL, respectively. These results suggest that it is unlikely that Pb in chocolate poses a significant health risk to consumers. Consequently, it does not appear that Proposition 65 warning labels are necessary for chocolate products. Our findings indicate that simply reporting the Pb content of a chocolate product is not sufficient to evaluate health risk; the health risks of Pb in chocolate should be evaluated in the context of estimated background exposures using predictive BLL models.

PS 2282 Effects of Serum Levels of Vitamin A and Its Precursors on Colorectal Cancer Mortality: An 18-Yr Follow-Up Study of a National Cohort

E. Afriyie-Gyawu[2], T. Ogunleke[2], C. O. Okoli[2] and J. Zhang[2]. [1]*Biostatistics, Georgia Southern University, Statesboro, GA and* [2]*Environmental Health Sciences, Georgia Southern University, Statesboro, GA.* Sponsor: E. Afriyie-Gyawu.

Vitamin A is essential for normal cell functioning. It is involved in cell growth, differentiation, and arrest, and eventually apoptosis which can inhibit tumor cell growth. A-carotenoids, precursors of vitamin A, are also important to maintain optimum physiological levels of this antioxidant. Colorectal cancer is the abnormal growth of cells in the colon or rectum. Of the cancers affecting both men and women, colorectal cancer is the second leading cancer killer in the US. One of the major risk factors for colorectal cancer is diet. Several studies have examined the association between vitamin A and various types of cancer. However, the relationship between serum vitamin A levels (and its precursors) and risk of colorectal cancer death is unclear. Thus, the goal of this study was to examine the relationship between serum levels of vitamin A (and A-carotenoid) and colorectal cancer mortality. A retrospective cohort study was conducted with 14,358 adults who participated in phase II of the National Health and Nutrition Examination Survey III (1991–1994) (NHANES III). This served as baseline and was correlated with the National Death Index database for a 15-yr (1991-2006) follow-up study. Hazard ratios (HRs) for all-cause and cancer-related deaths for individuals with high, medium, and low serum vitamin A and A-carotenoid levels were calculated using Cox Proportional Hazards Regression Model. Unadjusted HR of deaths associated with low serum levels of vitamin A were 1.84 (95%CI=1.02-3.30) and 1.00 (reference). After adjustment for multiple colorectal cancer risk factors including age and sex, the HR for colorectal cancer fatalities were 1.00 (reference) and 1.85 (95%CI=1.02-3.37). Adjusted HR for colorectal cancer death for A-carotenoid serum levels, using 3-level categorization and adjusted for fruits and vegetables were 2.92 (95%CI=1.49-5.71) and 1.89 (95%CI=1.03-3.45) for low and medium compared to high, respectively. Results indicate that there is a significant association between serum levels of vitamin A (and A-carotenoids) and colorectal cancer mortality. Further studies are warranted to determine the mechanism(s) of this phenomenon.

PS 2283 Aflatoxin and Fumonisin Co-Exposure in a Population From the Metropolitan Area of Monterrey, Mexico

S. E. Elmore[2], A. G. Marroquin Cardona[1], M. Bautista[1], P. Gonzalez-Barranco[1], R. M. Sanchez-Casas[1], C. Maki[2] and T. D. Phillips[2]. [1]*Biopharmacy Department, Faculty of Chemical Sciences, Universidad Autonoma de Nuevo Leon, Monterrey, Mexico and* [2]*Interdisciplinary Faculty of Toxicology, Veterinary Integrative Biosciences, Texas A&M University, College Station, TX;* [3]*Toxicology Department, Faculty of Veterinary Medicine, Universidad Autonoma de Nuevo Leon, Monterrey, Mexico.*

Aflatoxins (AFs) and fumonisins (FBs) are toxic metabolites produced by *Aspergillus* and *Fusarium* fungi, respectively. Both toxins commonly contaminate staple grains and cereals such as maize and have been associated with liver disease and cancer. In many high-risk populations, AFs and FBs biomarkers have been detected in humans; co-exposure to both toxins has not yet been reported in Mexico. In this study, participants with a history of maize consumption were recruited from 9 municipalities in Monterrey, Mexico. Urine samples from a total of 106 participants were analyzed for short-term biomarkers of toxin exposure. AFM1 and FB1 levels in urine were analyzed using UPLC/MS-MS in the ESI positive mode; MS-MS conditions were optimized for each compound. Briefly, AFM1 and FB1 were purified with Vicam AflaTest WB and FumoniTest immunoaffinity columns. Eluents containing the compounds were dried under N2 and resuspended prior to a 10 and 20 µL injection, respectively. AFM1 separation was achieved isocratically at a flow rate of 0.325 ml/min. The precursor ion was set to 329.0 Da and the two product ions were 273.0 and 259.1 Da. FB1 separation was achieved with a gradient at a flow rate of 0.4 mL/min; the precursor ion was set to 722.5 Da and the two product ions were 334.4 and 352.2 Da. External standards were injected daily and peak areas were quantified using Empower software. The geometric mean (95% CI) of urinary AFM1 and FB1 was 3.2 (2.3-4.0) and 27.44 (15.8-39.1) pg/mg creatinine and the frequency of samples above the detection limit was 68.9% and 71.7%, respectively. Of those participants with detectable levels of AFM1 or FB1, 55.7% were co-exposed to both toxins. Results from this study suggest that an urban population in Monterrey, Mexico has a high frequency of exposure to AFs and FBs, warranting further investigation into the potential for toxin interactions and impact on public health.

PS 2284 Serum Lycopene Levels and Prostate Cancer Mortality: An 18-Year Follow-Up Study of a National Cohort

E. Afriyie-Gyawu[2], D. Kanda[2] and J. Zhang[2]. [1]*Biostatistics, Georgia Southern University, Statesboro, GA and* [2]*Environmental Health Sciences, Georgia Southern University, Statesboro, GA.* Sponsor: E. Afriyie-Gyawu.

Lycopene is a carotenoid abundant in fruits and vegetables such as tomatoes, guava, etc. Several *in vitro* and animals studies have indicated that lycopene may have chemo-preventive effects for various cancers including prostate, skin, breast, lung and liver. However, reports on human trials linking lycopene and these chemo-preventive effects are inconsistent in their findings. The objective of this study was to determine whether serum lycopene levels influence prostate cancer mortality. A retrospective cohort study was conducted with 14,358 adults who participated in phase II of the National Health and Nutrition Examination Survey III (1991–1994) (NHANES III). This served as baseline and was correlated with the National Death Index database for a 15-yr (1991–2006) follow-up study. The primary outcome measure was mortality; and serum lycopene levels were categorized as low, medium and high. Other variables such as family income, educational attainment, marital status, race and ethnicity, alcohol consumption, cigarette smoking, self-reported health, cancer status at baseline, vegetable consumption, and fruit/juice consumption were also considered. Estimates of the crude and adjusted hazard ratios (HRs) of dying from prostate cancer and all cancers for individuals with different levels of serum lycopene were derived from Cox Proportional Hazard Regression models using the SAS software. Proportionality assumption of the models were checked by visual inspection of plots of log [-log(S)] against time. Results indicate that mortality from all cancers increased among individuals with low

versus high serum lycopene levels [Hazard ratio = 1.39 (1.19-1.63) for unadjusted rates] and [1.27 (1.08-1.49) for adjusted rates]. For prostate cancer mortality, however, no significant differences were detected between low versus high serum lycopene levels [Hazard ratio = 1.01 (0.57-1.79) for unadjusted rates] and [0.96 (0.53-1.73) for adjusted rates]. While lycopene reduces the risk of developing prostate cancer, this study suggests that serum lycopene does not prevent prostate cancer mortality. A major limitation of the study is that serum lycopene levels were measured only once during the study duration.

PS 2285 Co-Associations Between Selected Nutrients and Mercury in Wild-Harvested Fish of the Northwest Territories, Canada

B. D. Laird and E. S. Reyes. *University of Waterloo, Waterloo, ON, Canada.*

Because of seafood's nutrient density, the consumption of fish and shellfish can promote health in human populations. On the other hand, methylmercury, which occasionally reaches elevated concentrations in fish, can result in public health risks. To better understand the balance between these benefits and risks, the fatty acid profile, selenium content, and total mercury (HgT) levels within the muscle tissues of wild-harvested fish from three lakes in the Northwest Territories were characterized. As expected, HgT and omega-3 fatty acid (n-3 FA) levels differed from one species to another. For example, average HgT levels ranged from from 0.057 mg/kg (Cisco) to 0.551 mg/kg (Northern Pike) while average n-3 FA levels ranged from 101 mg/100 g (Burbot) to 1,689 mg/100 g (Lake Trout). In contrast, average Se concentrations were relatively similar between species. Consequently, Lake Whitefish, Cisco, and Longnose Sucker tended to have the highest n-3 FAs and Se levels relative to their HgT content. Interestingly, fish HgT sometimes covaried with the tissues' nutrient content. For example, significant negative associations were observed between Hg and n-3 FAs for Lake Trout (ρ = -0.937), Northern Pike (ρ = -0.619), and Walleye (ρ = -0.481). There were also significant negative co-associations between Se and Hg observed for Lake Whitefish (ρ = -0.818), Cisco (ρ = -0.685), and Northern Pike (ρ = -0.410). As such, for these species, samples with the greatest nutritional content tended to have lower levels of HgT. This work provides valuable information for the design of dose reconstruction models capable of refining public health messaging related to the balance of benefits and risks in seafood

PS 2286 Assessment of Liver Enzyme Changes Among NHANES Respondents and Association with Use of Dietary Supplements

S. Fallah, M. Ho, K. Ly, K. Musa-Veloso and L. A. Haighton. *Intertek Scientific & Regulatory Consultancy, Mississauga, ON, Canada.*

Dietary supplement use, particularly use of botanical supplements, has been implicated with liver toxicity in some cases. In the current research, the relationship between abnormal liver enzyme values, GGT, ALT, AST, and supplement use was explored using data from Day 1 of NHANES 2007-2012. Results for participants ≥16 years for whom blood tests had been conducted were analyzed. Normal reference ranges for GGT, ALT, and AST were applied and individuals with enzyme levels exceeding the upper end of the normal range were identified. The percentage of participants with abnormal enzyme levels who were also supplement users (users) were compared to the percentage of participants with abnormal enzyme levels who were not supplement users (controls). Results were analyzed separately for GGT, ALT and AST and by gender. Among males, 7.2% of users had abnormal GGT vs. 7.7% of controls, 7.8% of users had abnormal ALT vs. 11.2% of controls, and 15.4% of users had abnormal AST vs. 14.6% of controls. Among females, 12.7% of users had abnormal GGT vs. 11.6% of controls, 11.8% of users had abnormal ALT vs. 10.5% of controls, and 9.2% of users had abnormal AST vs. 6.9% of controls. In a second analysis, supplement users were further categorized as users of botanicals (botanical users) or as users of supplements other than botanicals (control = non-botanical supplement users), and the percentage of participants in each group with abnormal enzyme levels was compared. Among males, 6.1% of botanical users had abnormal GGT vs. 7.5% of controls, 7.9% of botanical users had abnormal ALT vs. 7.8% of controls, and 19.0% of botanical users had abnormal AST vs. 14.3% of controls. Among females, 11.0% of botanical users had abnormal GGT vs. 13.2% of controls, 12.3% of botanical users had abnormal ALT vs. 11.6% of controls, and 8.5% of botanical users had abnormal AST vs. 9.3% of controls. The effects of potential confounding factors which could impact these findings, including the use of prescription drugs, liver disease, and alcohol consumption, are being investigated.

PS 2287 A Tiered Approach to Safety Evaluation of Newly-Expressed Proteins in GM Crops

C. R. Fleming. *Dow AgroSciences, Indianapolis, IN.* Sponsor: R. Rasoulpour.

Thorough mammalian safety evaluation of genetically modified (GM) crops is a multifaceted process assessing dietary exposure to newly expressed proteins, health impacts of any changes in crop composition, and risk from herbicide residues or metabolites present on herbicide tolerant crops. Information in the form of history of safe consumption, lack of homology with known toxins or allergens, known mode of action, heat stability, and gastric and intestinal digestibility may be available to qualitatively assess protein dietary safety. Furthermore, current regulatory standards require acute and repeat dose toxicity studies on the protein, which to date have revealed no adverse effects, and a dietary exposure assessment. Often it is possible to conclude a reasonable certainty of no harm using qualitative information on exposure and safety, limiting the need for animal usage in *in vivo* toxicity testing. A tiered approach to assessing dietary risk from GM proteins will be introduced in this presentation that takes into account both qualitative and quantitative lines of evidence. This approach was applied to assess dietary risk for AAD-12 protein in soybean and AAD-1 protein in corn, demonstrating the value of this approach over a more prescriptive, box-checking approach. AAD-12 lacks homology to known allergens and toxins, is rapidly digested in simulated gastric fluid (SGF, <30 sec) and intestinal fluid (SIF, <5 min), and is deactivated by heat (50- 95°C). Soybean contains antinutrients and must be heated before consumption, so dietary exposure to active protein would be minimal, and the protein would be rapidly digested on ingestion, limiting systemic exposure. Therefore, reasonable certainty of no harm can be concluded for AAD-12 without the need for animal testing. AAD-1 similarly lacks homology to known allergens and toxins, is rapidly digested in SGF (<30 sec) and SIF (<1 min), and is deactivated by heat (50–95°C). This approach provides a harmonized, science-based framework for safety assessment of GM proteins, and reduces animal usage by up to 160 animals/protein under current guidelines, supporting the principles of the 3Rs for animal research.

PS 2288 Retrospective Assessment of Toxicology Data Needs to Support GRAS Determinations in Year 2015

J. Heilman and N. Tran. *Exponent, Inc., Washington, DC.*

The Generally Recognized as Safe (GRAS) determination process for food ingredients has been the focus of much recent media attention, in particular with the signing and ongoing implementation of the US Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA). Public awareness regarding the process has also increased. Since the publication of the first Redbook, the US FDA's cornerstone risk assessment guidance for food ingredients, the types of ingredients have broadened, and several updates to the Redbook have been drafted. The broadening array of potential food ingredients, changes in public perception of the GRAS process, and even subtle shifts in the political and regulatory climate make it important to understand the most recent expectations for supporting the safety of food ingredients. To this end, we evaluated all available GRAS notifications to the US FDA which reached closure during the year of 2015 in order to describe the current range of safety portfolios that have successfully supported GRAS determinations for which notifications to the US FDA resulted in letters of "no questions." Our analysis revealed that the core set of safety data that supported food ingredients under the GRAS notification process during 2015 was dependent on the type of ingredient, but tended to minimally include a 13-week subchronic toxicity study in rats, a bacterial reverse mutation assay, and an *in vitro* assay for chromosomal aberrations. Where clinical studies were available, rodent studies shorter than 13 weeks sometimes provided the basis for an acceptable daily intake of the ingredient, and in cases were genotoxicity studies were not clearly negative, repeat evaluations or *in vivo* studies were capable of dismissing genotoxicity concerns. Reproductive and developmental toxicity data were not typically included beyond the screening parameters commonly included in subchronic studies, and recovery phases were not consistently conducted in the key studies for safety determination. Margins of safety generally ranged from two to three orders of magnitude.

2289 Mild Endoplasmic Reticulum Stress Preconditioning Upregulates Gene Expression of Membrane Transporters

F. Usuki and M. Fujimura. *Basic Medical Sciences, National Institute for Minamata Disease, Minamata, Japan; Clinical Medicine, National Institute for Minamata Disease, Minamata, Japan.*
Sponsor: A. Naganuma.

We previously reported that mild endoplasmic reticulum (ER) stress preconditioning is a useful therapeutic intervention against methylmercury (MeHg) toxicity, the underlying mechanism being the induction of integrated stress responses (Scientific Reports 2013 doi: 10.1038/srep02346). Here, we investigated the effect of mild ER stress preconditioning on the expression of membrane transporters that potentially affect cellular influx and efflux of MeHg because ER stress preconditioning affected the intracellular Hg content. We examined mRNA expression of amino acid transporters LAT1, LAT3, and SNAT2, and an ATP-binding cassette (ABC) transporter cassette C subfamily 4 (ABCC4) which is related to the efflux of glutathione conjugates. Total RNA was extracted from MeHg-susceptible myogenic cell line which was preconditioned with low concentration (0.1-0.3μg/ml) of an inhibitor of ER Ca2+-ATPase, thapsigargin (TPG) 16 h before the treatment with MeHg. Then samples were analyzed by real-time PCR. The results demonstrated that mild ER stress preconditioning upregulated gene expression of all of these transporters and that the upregulation of ABCC4 mRNA was much higher than the others. The intracellular Hg content reflected the changes in the expression of these transporters. Mild ER stress preconditioning induces integrated stress responses, including phosphorylation of eukaryotic initiation factor 2 alpha (Eif2α), accumulation of activating transcription factor 4 (Atf4), upregulation of stress-related proteins, and suppression of nonsense-mediated mRNA decay (NMD) activity. Knockdown study of Atf4, phosphorylation kinase of Eif2α, and NMD factors suggested that the upregulation of LAT3 was due to NMD suppression but the upregulation of SNAT2, LAT1, and ABCC4 transporters might be related to other factors.

2290 Cigarette Smoke Exposure Leads to the Recruitment of NLRP10 and NLRP12 in Lipid Raft Entities

D. P. Singh and S. Batra. *Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.*

Lipid rafts, the detergent-insoluble micro-domains in the plasma membrane of the cells and contribute to diversity of cellular processes. Membrane recruitment of some NOD-like receptor family members (NOD1 and NOD2) is important for their response against a wide array of adjuvants. Our preliminary results showed important role of NLRP10 and NLRP12 in secondhand smoke induced inflammatory responses in C57Bl/6 mice. We hypothesized that lipid rafts regulate NLRP10 and NLRP12 (NLR family members) mediated inflammatory responses against cigarette smoke (CS) challenge. Our *in silico* studies showed the possibility of molecular interactions between NLRP10 and NLRP12 with lipid raft proteins (caveolin-1 and flotillin-1). We conducted *in vitro* experiments using THP-1 and RAW264.7 murine macrophages and challenged them with CS condensate (Murty Pharmaceuticals). We observed increase in the expression of both NLRP10 and NLRP12, and lipid raft proteins caveolin-1 and flotillin-1 in response to CSE challenge. Both NLRP10 and NLRP12 were observed to localize in lipid raft entities in CS challenged RAW 264.7 macrophages and THP-1 cells. Further, we also observed CS mediated induction of inflammatory response. However, we were unable to detect any significant interaction between NLRP10 and NLRP12 by confocal imaging. Our findings show that lipid rafts regulate NLRP10 and NLRP12 mediated inflammatory responses against CS challenge. Our findings are novel and may lead to development of new therapeutic strategies.

2291 An Imaging-Based RNA-Interference Screen Reveals Novel Key Regulators of the Drug-Induced Endoplasmic Reticulum Stress Response

M. Niemeijer, G. Callegaro and B. van de Water. *Toxicology, Leiden University, Leiden, Netherlands.*

Drug induced liver injury (DILI) is one of the main reasons for drug withdrawal from the market and is a major concern in drug development. To accurately predict drug-induced hepatotoxicity, it is essential to identify the underlying cause of DILI. Various hepatotoxicity-inducing drugs, such as diclofenac, are known to induce perturbations to the endoplasmic reticulum (ER) leading to the activation of the unfolded protein stress response (UPR). Under sustained ER stress conditions, apoptotic pathways will be activated leading to hepatotoxicity; CHOP is essential in this process. Therefore, it is crucial to identify novel key regulators of the ER stress response to obtain a better understanding of the development of DILI. First, we have established HepG2 BAC-GFP reporters for the UPR, including a GFP-CHOP. Various DILI compounds do activate the BAC-GFP-CHOP reporter in a concentration dependent manner. Next, to identify novel regulators of the UPR response, a high-content imaging-based RNAi screen of the druggable genome targeting 3457 genes was performed in these GFP-CHOP reporter cells. The effect of knockdown on GFP-CHOP expression after 16 hours exposure to tunicamycin was evaluated. After validation of the hits by using four individual siRNAs, potential regulators could be identified either down- or up-regulating GFP-CHOP expression during ER stress. For instance, upon knockdown the genes UFD1L and RBM25 drastically increased the GFP-CHOP expression. RBM25 regulates apoptotic signaling through alternative splicing, indicating that the spliceosome could play a crucial role during the ER stress response. The next step focuses on validating the hits with other DILI compounds activating the UPR, as well translating this knowledge to primary human hepatocytes. In conclusion, our RNAi screen allowed the identification of novel regulators of the drug-induced ER stress response and will further shape our understanding and prediction of DILI liabilities as well as individual susceptibilities.

2292 Regulation of FGF15 Expression in Mouse Intestine

X. Fan[2], K. Jia[2], L. Ding[2], X. Ding[1] and Q.-Y. Zhang[2]. [1]*College of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY and* [2]*Wadsworth Center, New York State Department of Health and School of Public Health, State University, Albany, NY.*

Fibroblast growth factor 15 (FGF15) and its human ortholog FGF19 are enterohepatic signaling molecules synthesized primarily in the ileum. They play important roles in regulating bile acid homeostasis, by suppressing the expression of hepatic CYP7A1, a rate-limiting enzyme in bile acid synthesis. FGF15/19 is known to be directly regulated by FXR and VDR. However, it is still poorly understood how these nuclear receptors contribute to FGF15/19 regulation *in vivo*, and whether FGF15/19 regulates P450 gene expression in the intestine through an autocrine or paracrine mechanism. In the present study, we explored the mechanism of regulation of FGF15 expression in the intestine by various nuclear receptors using a pharmacological approach, and found that, in addition to FXR and VDR, the glucocorticoid receptor (GR) can also regulate FGF15 expression. In further studies utilizing an intestinal epithelium-specific P450 reductase knockout (IE-Cpr-null) mouse model, we observed that FGF15 expression was reduced in IE-Cpr-null mice, in which FXR activity was suppressed, but VDR activity was increased. This data suggested that FXR plays a more important role than VDR does in the activation of intestinal FGF15 expression *in vivo*. Furthermore, we confirmed that FGF15 can play a direct role in the regulation of P450 (CYP3A, CYP2B, and CYP2C) expression in *ex vivo* cultures of mouse ileum. Overall, our results indicate a novel role of GR in FGF15 regulation, and provide the basis for further mechanistic studies of physiological regulation of FGF15/19 expression in the intestine. (Supported in part by NIH grant GM082978)

2293 Modulation of NF-κB Activation Is a Major Contributor to Drug-Induced Liver Injury (DILI)

S. Huppelschoten, S. Wink, A. Essing and B. van de Water. *Toxicology, Leiden University, Leiden, Netherlands.*

Drug-Induced Liver Injury (DILI), in particular idiosyncratic DILI, remains a major problem in the clinic and for drug development. Currently, the very heterogenic group of chemicals inducing idiosyncratic DILI cannot be predicted *in vitro*, *in vivo* or in clinical trials. Previously, we and others have demonstrated synergism between some idiosyncratic DILI compounds and pro-inflammatory cytokines, e.g. TNFα, towards cell death. TNFα activates NF-κB, a transcription factor regulating immune reactions, cell growth and cell death. Here, we investigated how DILI compounds affect NF-κB activity. We established a panel of Bacterial Artificial Chromosome (BAC)-GFP reporters in HepG2 cells based on the major components of the NF-κB signaling pathway: the negative regulator of NF-κB, I-κBα the NF-κB subunit RelA; and NF-κB target genes, ICAM1 and A20. We assessed the effect of a large series of ~100 DILI compounds on TNFα-induced NF-κB signaling with live cell confocal microscopy and quantified the dynamic responses at the single cell level. Various DILI compounds affected TNFα-induced signalling, which was often associated with a direct effect on NF-κB activation; in several cases this effect co-occurred with synergistic cell death. The effect of disturbed TNFα-induced NF-κB activation by DILI compounds was further investigated at the level of NF-κB target gene induction using Fluidigm PCR. Intriguingly, DILI compounds that do affect TNFα-mediated sig-

nalling, themselves induced the expression of various direct NF-κB target genes, including the NF-κB family members RelB and NFKB2. We hypothesize that the induction of various NF-κB target genes by DILI compounds may negatively control the prototypical NF-κB signalling by cytokines, but does not prevent the cell death signalling route activated by TNFR engagement. This work was supported by the IMI MIP-DILI project (grant agreement 115336).

2294 Evaluation of Polycyclic Aromatic Compound Toxicity Using Metabolically-Competent *In Vitro* Liver Models

P. E. Dunlap, S. Ramaiahgari, E. J. Tokar, M. Fenton, S. Waidyanatha, M. DeVito, W. A. Boyd, C. Rider and S. S. Ferguson. *NTP, NIEHS, Research Triangle Park, NC.*

Polycyclic aromatic compounds (PACs) are a frequent source of contamination in the environment (e.g. Deep Water Horizon Spill in the Gulf of Mexico) with the potential to affect human health. As part of the NTPs Polycyclic Aromatic Compound Mixtures Assessment Program (PACMAP), *in vitro* liver models were developed to assess the toxicity potential of this class of compounds. Many PACs are known to require metabolic activation to exert their toxicity (e.g. genotoxicity), therefore *in vitro* liver models with physiologically-relevant levels of xenobiotic metabolism competence are required to more effectively evaluate the toxicity potential of PACs in humans. Twenty four PACs were chosen for these evaluations using HepaRG which have recently emerged as a metabolically-competent *in vitro* liver model for toxicology research. In this report, we optimized 2 sets of culture conditions: 1) maximal constitutive metabolic competence and 2) inducible liver enzymes. Under maximal metabolic competence, cytotoxicity was assessed via mitochondrial function (MTS assays), cytosolic membrane integrity (LDH assays), and cellular morphologies for the set of 24 PACs. Multiple PACs produced changes in cell morphology consistent with cytotoxicity that were supported by MTS and LDH assays. Under inducible conditions 5 PACs produced statistically significant increases in LDH leakage (Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno[1,2,3,c,d]pyrene, Benzo(k)floranthene, Dibenz(a,c)anthracene) that were supported with cell morphology assessments. Future -efforts will investigate the relationships between PAC chemical structures and their responses in these metabolically-competent HepaRG cultures.

2295 Protective Effect of Sodium Tetrasulfide on Covalent Modification of Cellular Proteins and Cellular Toxicity Mediated By 1,4-naphthoquinone, an Atmospheric Electrophile

R. Hirose, T. Unoki, Y. Shinkai, Y. Abiko and Y. Kumagai. *Faculty of Medicine, University of Tsukuba, Tsukuba, Japan.*

1,4-Naphthoquinone (1,4-NQ) is found as an atmospheric electrophile that is readily able to modify cellular proteins through their cysteine residues, resulting in cytotoxicity. We found that reactive sulfur species (RSS) such as glutathione persulfide (GS-SH) and its polysulfide (GSSSG) with high nucleophilicity are endogenously produced in cells (Ida T et al. PNAS 2014), suggesting that reactivity of 1,4-NQ could be terminated by RSS though its sulfur adduct formation. Exposure of primary mouse hepatocytes to 1,4-NQ caused covalent modification of the cellular proteins as determined by immunoblot analysis with specific antibodies against 1,4-NQ and substantial cell death as evaluated by MTT assay in a concentration-dependent manner. Surprisingly, simultaneous treatment of 1,4-NQ with sodium tetrasulfide (Na2S4) as a model for polysulfide completely blocked 1,4-NQ-mediated protein modification, thereby diminishing cellular toxicity, indicating a possibility of interaction of 1,4-NQ with this polysulfide. Consistent with this notion, LC-MS/MS and FT-ICR-MS analyses revealed that incubation of 1,4-NQ with Na2S4 resulted in formation of sulfur adducts corresponding to 1,4-NQH2-SOSH, 1,4-NQ-SO-SO-1,4-NQH2 and 1,4-NQ-SO-1,4-NQ. These results suggest that blockage of 1,4-NQ-mediated covalent binding to cellular proteins and cytotoxicity by polysulfides such as Na2S4 appears to be attributable to the sulfur adduct formation. Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (#25220103 to YK).

2296 CRM1 (Exportin 1) Inhibitors, Leptomycin B and Curcumin, Alter CRM1's Subcellular Distribution

K. Rahmani and D. A. Dean. *Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY; Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, NY.* Sponsor: J. Finkelstein.

Chromosome maintenance region 1 (CRM1, Exportin 1) belongs to the karyopherin group of transport proteins and has a well established function as a nuclear export receptor that mediates export through the nuclear pore complex (NPC). CRM1 binds to nuclear export signals (NES) and is required in nucleocytoplasmic transport of a large variety of proteins including transcription factors, cell cycle regulators, and RNP complexes. CRM1 activity is increased in various cancers including cervical, pancreatic, ovarian, and gliomas, where it exports several tumor-suppressive factors from the nucleus allowing for cell cycle disregulation. Thus CRM1 is an obvious target for the development of chemotherapeutic agents that can inhibit CRM1 function resulting in an increase in the presence of tumor-suppressive proteins within the nucleus. Leptomycin B (LMB), the first specific inhibitor of CRM1 identified, binds covalently to cysteine 528 in the NES binding region of CRM1 leading to the inhibition of protein nuclear export. Due to the toxicity of LMB *in vivo*, several other CRM1 inhibitors are currently under investigation for use as chemotherapeutic agents. Curcumin, an anti-inflammatory compound isolated from the turmeric plant has been shown to inhibit CRM1 nuclear export function in HeLa cells. We have found that LMB, as well as curcumin, cause CRM1 cargo proteins to accumulate in the nucleus and also causes subcellular redistribution of CRM1 from the nucleus into the cytoplasm in A549 cells, an aleveolar epithelial adenocarcinoma cell line. This redistribution was observed using nuclear cytoplasmic fractionation of cell lysates as well as immunofluorescence. The redistribution of CRM1 is observed as early as 30 min to 1 hour (LMB and curcumin, respectively) with no change in total cellular CRM1. Decreasing the nuclear availability of CRM1 likely contributes to the accumulation of CRM1 cargo proteins in the nucleus, suggesting that this new mechanism of action can be utilized for drug development while decreasing the toxic side effects of inhibiting CRM1 activity.

2297 Liver Enzyme Inducibility of Polycyclic Aromatic Compounds in Metabolically-Competent Cultures of HepaRG

S. Ferguson and S. Ramaiahgari. *DNTP, NIEHS, Durham, NC.*

Polycyclic aromatic compounds (PACs) are common constituents of environmental pollution (Deepwater Horizon Gulf Oil Spill), yet significant deficiencies exist in our knowledge of human health hazard potential or mechanistic understanding that driver their toxicological effects. PACs have frequently been associated with metabolically-activated toxicity, yet most *in vitro* research with PACs employ culture models lacking physiologically-relevant levels/proportions of xenobiotic metabolism. In this study, we evaluated the temporal dynamics of metabolic competence with cryopreserved HepaRG cells observing a substantial (5 to 10-fold) loss of initial activity (suspensions) that was maintained with induction media supplements and could be fully recovered after ~10-days with metabolism media. Matrigel overlay further increased metabolic competence. Twenty four (24) PACs were assessed in concentration-response liver enzyme induction in HepaRG cultures (48hr exposures) monitoring mRNA expression (CYP1A2, CYP2B6, CYP3A4, HMGCS2, ABCB11) and liver enzyme activity (CYP1A2, CYP2B6, CYP3A4). From these data, 15 of the 24 PACs produced >10-fold induction of CYP1A2, 1 for CYP3A4, and multiple compounds with 3-5 fold induction of CYP2B6. 5 of the PACs produced highly potent responses with EC50s < 0.78μM (lowest concentration evaluated). Interestingly, 'pan' decreases in metabolism/transport gene expression and enzymatic activities were observed with 12 of 24 PACs at markedly lower concentrations than observed with LDH leakage assays. Taken together, these data suggest the majority of the PACs activated AhR to induce CYP1A2, a few were CAR/PXR activators (increased CYP2B6/3A4), and none increased FXR or PPARα targets. The highly potent 'pan' decreases in metabolism/transporter expression will be further evaluated (i.e. inhibition, ROS, high content imaging assays) to evaluate the potential mechanisms of these adaptive responses that did not appear overtly cytotoxic after 48 hrs.

2298 1,4-Naphthoquinone-Mediated Activation of the HSP90/HSF1 Signal Transduction Pathway and Its Modulation by Reactive Sulfur Species in Human A431 Cells

T. Unoki, Y. Shinkai, L. Sha and Y. Kumagai. *Faculty of Medicine, University of Tsukuba, Tsukuba, Japan; Human Biology Program, University of Tsukuba, Tsukuba, Japan.*

1,4-Naphthoquinone (1,4-NQ) is found as an atmospheric electrophile that is readily able to modify protein nucleophiles. Covalent modification of cellular proteins by electrophiles affects electrophilic signal transduction and cell damage. We recently found that reactive sulfur species (RSS) with high nucleophilicity are endogenously produced in cells (Ida T et al., PNAS 2014), and that a model polysulfide sodium tetrasulfide (Na2S4) blocks 1,4-NQ-mediated covalent binding to cellular proteins and cytotoxicity through its sulfur adduct formation. In this study, we explored whether 1,4-NQ could activate heat shock protein 90 (HSP90)-heat shock factor 1 (HSF1) signaling that might be repressed by RSS. HSP90 was covalently modified by 1,4-NQ as determined by UPLC-MSE analysis, pull-down assay, and BPM-labeling assay. 1,4-NQ-mediated modification of HSP90 induced dissociation of HSF1 from HSP90, and allowed its translocation to the nucleus, resulting in upregulation of HSP gene transcription levels, such as HSPA6, HSP40, HSP90, and HSP105 in A431 cells. The increase of 1,4-NQ-induced cytotoxicity by HSF1 knockdown confirmed that activation of the HSP90/HSF1 signaling plays a role in the cell survival during 1,4-NQ exposure. 1,4-NQ-induced nuclear translocation of HSF1 was markedly blocked by Na2S4 in A431 cells. In contrast, knockdown of cystathionine β-synthase (CBS), an RSS generating enzyme, enhanced 1,4-NQ-induced nuclear translocation of HSF1. These results suggest that 1,4-NQ-mediated activation of the HSP90/HSF1 signal transduction pathway is negatively modulated by RSS in A431 cells. Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (#25220103 to YK).

2299 Characterization of Ligand-Selective Activation of the Human and Mouse Aryl Hydrocarbon Receptors

S. C. Faber, A. Soshilov and M. S. Denison. *Environmental Toxicology, University of California, Davis, Davis, CA.*

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that can be activated by structurally diverse chemicals. Although the AhR is highly conserved across species, a dramatic range of species- and tissue-specific toxic and biological effects produced following AhR-dependent activation, suggests ligand-dependent differences in AhR action. To examine the mechanisms responsible for the ligand-selective and species-specific differences in AhR ligand binding, we examined the ability of structurally-diverse chemicals to activate the mouse and human AhR *in vitro* and in cells in culture. Human and mouse AhR proteins were synthesized *in vitro* and the ability of these diverse ligands to stimulate AhR DNA binding determined using gel retardation analysis, and to stimulate AhR-dependent luciferase gene expression confirmed using stably transfected mouse and human CALUX cell lines. Indirubin, ITE and 5F-203 were identified as ligands that were more selective for the human AhR compared to mouse. ITE and indirubin enhanced human AhR-dependent reporter gene induction and DNA binding to levels significantly greater than the maximal response produced by TCDD, and indirubin and 5F-203 were more potent than TCDD at inducing AhR-dependent gene expression in human cells. Compared to the human AhR, not only were TCDD and TCDF 10-fold more potent in activating mouse AhR-dependent gene expression, but they also stimulated a greater amount of DNA binding by the mouse AhR. Together, these data identifies AhR ligands with greater selectivity for the human AhR compared to the mouse AhR, and suggests that differences in species-specific endpoints may be influenced by altered ligand-selective differences in the efficiency of AhR transformation and gene transcription. This work is funded by the NIEHS (ES07685 and ES04699 (MSD) and Predoctoral fellowship T32ES007059 (SCF).

2300 Characterizing Novel Alternative Splicing Isoforms of Critical Drug-Processing Genes in Mice

D. H. Cowles[1,2], C. Y. Li[1] and J. Y. Cui[1]. [1]*Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and* [2]*Department of Environmental Sciences, Louisiana State University, Baton Rouge, LA.* Sponsor: J. Cui.

Alternative splicing of critical drug-processing genes may lead to altered toxicological responses. The goal of this study was to confirm the presence and further determine the regulation of selected novel transcripts encoding drug-processing genes in mice. The presence of the novel transcripts was confirmed by End-point PCR, gel electrophoresis, and sequencing. Regarding the tissue distribution of the known and novel alternative splicing transcripts, key metabolic organs including the liver, kidney, and intestine, were collected from wild-type adult male mice. Regarding the hepatic ontogeny of these transcripts, livers were collected from male and female mice at 12 developmental ages. Regarding the regulation of these transcripts by xenobiotics, adult male conventional and germ-free mice were treated with the environmental toxicant polybrominated diphenyl ether 47 (PBDE-47) once daily for 4 days, and livers were collected on the 5th day. Isoform-specific expression of the genes was determined using RT-PCR. In general, most transcripts were expressed most highly in the liver, and tissue-specific splicing events were observed for aldehyde dehydrogenase (Aldh) 3a2 (preferably expressing the intron-retained novel transcript in kidney and colon), as well as kynureninase (Kynu) (preferably expressing the exon 8-skipped novel transcript in the kidney). During liver development, there was a gradual increase in the transcript expression, which peaked between adolescence and adulthood. The novel isoforms of Aldh3a2, cytochrome P450 (Cyp) 2d10, and Kynu (exon 6 skipping) were consistently lower than the known isoforms, whereas the known and novel isoforms of Cyb5a, Kynu (exon 8 skipping), and solute carrier (Slc) 17a2 were expressed at similar levels. In contrast, the novel transcript of Slc22a23 (exon 9 skipping) was the only expressed isoform in all tissues and throughout liver development. PBDE-47 up-regulated both the known and novel transcripts of Sult5a1, but down-regulated Slc17a2, in a gut microbiota-dependent manner. In conclusion, the alternative splicing events of these drug-processing genes display high tissue- and age-specificities; however, response to xenobiotic exposure is consistent for both the known and novel isoforms.

2301 Mechanistic Insight into Metformin-Mediated Repression of the Bile Salt Export Pump (BSEP; ABCB11)

B. Garzel[4], H. Yang[2], S. Heyward[1], L. Zhang[3], S.-M. Huang[3], J. E. Polli[4], J.-P. Raufman[4] and H. Wang[4]. [1]*BioreclamationIVT, Baltimore, MD;* [2]*China National Center for Food Safety Risk Assessment, Beijing, China;* [3]*US FDA, Silver Spring, MD and* [4]*University of Maryland, Baltimore, Baltimore, MD.*

Drug-induced cholestatic liver injury is a toxic side effect for a number of FDA-approved drugs, though it's cause is still not completely understood. Current research regarding drug-induced cholestasis primarily focuses on the inhibitory capacity of drugs for the bile salt export pump (BSEP) while often overlooking the importance of drug-induced gene repression. Metformin, a widely used type 2 diabetic drug, has been linked to cholestatic liver injury in a number of case reports; however metformin has no inhibitory effect on BSEP activity. In this report, human primary hepatocytes were used to assess metformin-mediated alteration of BSEP gene expression and function. The underlying mechanisms were further analyzed in cells expressing constitutively active (CA)- or dominant negative (DN)-adenosine monophosphate-activated protein kinase (AMPK), farnesoid x receptor (FXR), or nuclear factor (erythroid-derived-2)-like 2 (NRF2). Our data indicate that metformin can potently repress BSEP expression in human primary hepatocytes in a concentration-dependent manner. Interestingly, although metformin did not directly interact with BSEP, metformin-mediated BSEP repression resulted in decreased efflux of taurocholate in human primary hepatocytes. We have found that increased activity of AMPK, whether induced chemically or with adenoviral infection, leads to decreased BSEP gene expression. On the other hand, metformin appears to disrupt NRF2 transactivation of BSEP, as demonstrated in cell-based luciferase assays. Overall, these results indicate that BSEP function can be altered through gene repression without direct inhibition of BSEP activity, providing important information regarding the development of cholestatic liver injury, particularly under chronic drug exposure. Our results also indicate AMPK activation and NRF2 play a role in drug-induced BSEP repression. This information could be useful in screening for cholestatic potential of new drugs.

2302 Lead Acutely Induced Fibroblast Growth Factor 21 in Mouse Liver and Hepatocytes

S. G. Vispute, Y. Le, L. Chen and X. Cheng. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Heavy metals such as arsenic, cadmium, lead, and nickel are ubiquitously distributed but have no essential role in maintaining cellular homeostasis. Heavy metals exert toxicities in multiple organs and generally lead to chronic disease. It has been reported that heavy metal-induced acute toxicities are often due to enzymatic inhibition or oxidative stress. However, the molecular mechanisms and critical signaling pathways underlying the toxicity of heavy metals still remains elusive. Upon absorption, more than 33% of absorbed lead accumulates in liver, which alters hepatic xenobiotic metabolism, cholesterol metabolism and cell proliferation. Fibroblast growth factor (Fgf) 21 is a metabolic regulator that plays an important role in the maintenance of glucose, lipid, and energy homeostasis. Recent studies also demonstrated that gain of Fgf21 function can attenuate chemical-induced toxicities, such as of acetaminophen, alcohols and dioxins. In the present studies, we investigated whether heavy metals can regulate Fgf21 mRNA expression in mouse liver as well as in cultured mouse and human hepatoma cells. Amongst various heavy metals, lead, in the form of lead acetate, upregulated Fgf21 mRNA expression in mouse liver and Hepa1c1c7 hepatoma cells in a dose/concentration-dependent manner. In addition, in Hepa1c1c7 cells, lead induced Fgf21 mRNA expression after 6 and 12 hours post-treatment. However, such Fgf21 induction by lead was attenuated after 24 and 48 hours post-treatment. Therefore, in cultured Hepa1c1c7 cells, lead acetate only acutely increased Fgf21 mRNA expression. Moreover, lead did not alter the Fgf21 mRNA expression in human hepatoma (Hep3B, HepG2, or Huh7) cells. Taken together, lead induced Fgf21 expression in mouse liver and mouse hepatoma cells, but not human hepatoma cells.

2303 Genomic Analysis Directs a Multidisciplinary Approach to Define Toxicity for Inhalation Phosphine Poisoning

R. J. Lewandowski. *USAMRICD, Gunpowder, MD.* Sponsor: A. Sciuto.

Phosphine is a pesticide and rodenticide fumigant. The poisonous gas is released during hydrolysis of aluminum, magnesium, and zinc phosphide pellets as they contact water. Widespread use and ease of access to the pellets have increased concern over the potential safety threat to civilian populations. Acute phosphine exposure causes mortality via circulatory or respiratory failure; however, the pathological mechanism of death has been difficult to define. Phosphine exposure induces a variety of physiological changes including cellular poisoning and oxidative stress, cardiotoxicity, vascular collapse, gastrointestinal and pulmonary toxicity, hepatic disturbances, neurological toxicity, electrolyte imbalance, and metabolic disturbances. Diverse symptom presentation and inconclusive pathological analysis have initially provided little direction in determining the mechanism of phosphine toxicity. In this study, gene expression was used in the absence of definitive pathologic findings to direct ultrastructural pathologic evaluation and to identify molecular mechanisms of toxicity. Male Sprague-Dawley rats were exposed to phosphine gas in plethysmography chambers, and euthanized at 1, 3, 6, and 24 hours post-exposure. Heart, lung, kidney, and brain tissue was removed and isolated RNA was processed for hybridization to Affymetrix array plates. Raw signal intensities were imported into Partek Genomic Suite for principal component analysis and analysis of variance. Gene lists exhibiting significant expression changes were imported into Ingenuity Pathway Analysis software to identify canonical pathways, biological functions, diseases and disorders, and gene networks altered by phosphine exposure. Pathways highlighting cardiac abnormalities prompted in-depth examinations of phosphine-exposed heart tissue to reveal ultrastructural myocardial damage and mitochondrial membrane disruption. This was further substantiated through analysis of real-time physiological data from implanted rats that revealed changes in the ST interval and the QRS complex and fluctuations in blood pressure. Future objectives will utilize gene expression data to examine molecular aspects contributing to observed dose-dependent toxicity, and to guide drug selection for therapeutic development.

2304 The Gene Expressions of Ah Receptor Pathways Are Pervasively Regulated by Long Noncoding RNA Malat1

J. Chen[3], S. Ke[3], G. Wang[3], J. Cai[3], D. Li[1] and Y. Tian[3]. [1]*China Agricultural University, State Key Laboratory of Animal Nutrition, Beijing, China;* [2]*Vet Physiology and Pharmacology, Texas A&M University, College Station, TX and* [3]*Veterinary Integrative Biosciences, Texas A&M University, College Station, TX.* Sponsor: Y. Tian.

Malat1 (metastasis associated lung adenocarcinoma transcript 1) is a highly conserved long noncoding RNA and has been shown to regulate global gene expression by interacting with the spliceosome. The malat1 null mice are viable and fertile in laboratory conditions. There has been no reports on the stress responses upon exposure to the environmental chemicals. In this study, we investigated the role of malat1 in regulating AhR pathway in liver cell lines as well as the hepatocytes isolated from malat1 null mice. We performed transcriptomic analysis of AhR-regulated pathways with RNA-seq and found significant differential gene expression associated with malat1 deficiency. Dioxin treatment of the hepatocytes regulated expression changes in similar sets if genes. However, the magnitudes of dioxin responsive genes differed greatly between malat1 null and wild type hepatocytes with the "core" AhR-regulated mRNA levels augmented by 2-3 fold greater in malat1 null hepatocytes than the hepatocytes from wild type mice. These genes include cyp1a1, cyp1a2, cyp1b1, nqo1 and tiparp. The AhR activation induced cyp1a1 transcription faster at earlier time points suggesting different kinetics of transcription. The dioxin-induced fold induction of ethoxyresorufin-O-deethylas (EROD) activity of malat1 null and wild type hepatocytes were comparable despite of the large differences in the transcript levels suggesting a role in post-transcriptional regulation by malat1. The discordance may be due to the RNA processing defects in malat1 null mutant mouse which generate nonfunctional mRNA of CYP1A family, and consistent with this notion we have observed RNA processing abnormalities such as "intron retention" in the malat1 null mouse hepatocytes.

2305 Identification of a Conserved Long Noncoding RNA Elevated in Response to the Xenobiotic Activation of the Aryl Hydrocarbon Receptor

G. R. Garcia. *Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

The aryl hydrocarbon receptor (AHR) is necessary for proper vertebrate embryonic development, but can be inappropriately activated by a diverse group of xenobiotics including chlorinated dioxins, biphenyls, and polyaromatic hydrocarbons. To identify downstream AHR targets, RNA was isolated from 48hpf zebrafish embryos (exposed to 10uM 7,12-benz[a]anthracene quinone) and sequenced using paired-end Illumina sequencing. One of the most elevated transcripts was a novel long noncoding RNA (lncRNA), which we dubbed Sox9b-lncRNA due to its adjacency to the Sox9b gene (ortholog of human Sox9). AHR-dependent repression of Sox9, a conserved transcriptional regulator required for proper vertebrate development, is well established in both mammals and fish; however, the exact mechanism of repression is unknown. We sequenced the predominant Sox9b-lncRNA transcript, which contains 3 exons and is 467nt long. The genomic architecture of this lncRNA is conserved in mammals, suggesting similar mechanisms of regulation. The (mammalian and fish) Sox9-lncRNA promoter also contains putative AHR response elements, and a potential lncRNA binding site has been identified in the Sox9b promoter. We hypothesize that the conserved Sox9-lncRNA is a direct AHR target gene that transcriptionally represses Sox9 upon induction by strong AHR ligands to produce target organ specific toxicity. In support of this hypothesis, developmental exposure to 1nM TCDD in AHR2-null zebrafish lines showed Sox9b-lncRNA induction requires AHR2. Additionally, whole mount *in situ* hybridization showed that Sox9b mRNA and Sox9b-lncRNA are expressed in overlapping tissues during development. Furthermore, Sox9b-lncRNA morphants exposed to 1ng/mL TCDD, have a significant increase in Sox9b expression, suggesting a relief in repression. This research was supported by the SRP Grant P42 ES016465, NIEHS Core Center Grant P30 ES000210, and the NIEHS Training Grant T32 ES007060.

2306 Erk-Creb Pathway Suppresses Glutathione-S-Transferase Pi Expression in Zebrafish (*Danio rerio*) Embryos

J. Hrubik, B. Glisic and N. Andric. *Department of Biology and Ecology, University of Novi Sad Faculty of Sciences, Novi Sad, Serbia.*

Transcriptional activation of phase II enzymes including glutathione-S-transferases (GSTs) is important for redox regulation and defense from xenobiotics. The role of extracellular signal-regulated kinase (Erk) in regulation of Gst pi class (Gst Pi) expression has been described using adult mammalian cells, but is unknown in embryogenesis. To reveale the role of Erk signaling in the regulation of different Gsts mRNA during embryogenesis, zebrafish embryos were exposed to pharmacological inhibitors of Erk (U0126 and PD98059). Inhibitors were added at 2 and/or 5 hours post fertilization (hpf). Moreover, phorbol 12-myristate 13-acetate (PMA, 200 ng/mL) as inductor of Erk, and forskolin (50 µM), as inductor of Pka/Creb were applied at 24 hpf. Western blot and gene expression analyses were conducted at 12, 30, 48, and 96 hpf. PD98059 (25 and 50 µM) was embryotoxic (mortality rate: 68% and 78%, respectively) and therefore could not be used as an Erk signaling inhibitor. Mortality rate was 10% in the U0126-exposed embryos. Using U0126, we provide novel evidence that Erk signaling acts as a specific suppressor of gstp1-2 mRNA during early embryogenesis. Inhibition of the basal Erk activity enhanced gstp1-2 mRNA expression during transition from blastula to the segmentation stage (U0126, 100 µM) and from pharyngula until the hatching stage (U0126, 10 µM). Increase in the Erk activity by PMA attenuated gstp1-2 mRNA expression in embryos. Activation of cAMP/Creb pathway by forskolin prevented gstp1-2 expression, whereas U0126 suppressed Creb phosphorylation, thus setting up Creb as a proximal transmitter of Erk inhibitory effect. Collectively, these findings suggest that Erk-Creb pathway exerts suppressive effect on gstp1-2 mRNA in a narrow developmental window. This study also provides a novel link between Erk and gstp1-2 expression, setting apart a possible differential regulation of gstp1-2 in adult and embryonic cells. Financial support: MESTD of the Republic of Serbia (173037).

2307 Comparative Analysis of Nrf2 Target Genes Reveals Novel Functions

S. E. Lacher[1], J. Lee[1], X. Wang[2], M. Campbell[2], D. Bell[2] and M. Slattery[1]. [1]*Biomedical Sciences, University of Minnesota Medical School, Duluth, MN and* [2]*Environmental Genomics Section, NIEHS, Research Triangle Park, NC.*

The transcription factor NRF2 is a master regulator of the response to oxidative stress. We used genome-wide chromatin immunoprecipitation and transcriptome profiling to identify NRF2 target genes conserved from fly to human. This ancient network consists of the expected antioxidant genes, such as HMOX1, as well as genes related to proteolysis, metabolism, and a number of less expected genes. Finding genes involved in proteostasis was consistent with known NRF2 functions; however, their prominence on this list and the fact that NRF2 has the potential to regulate proteasomal activity at multiple levels - 19S subunit (PSMA3, PSMA4), 20S subunit (PSMD4, PSMD6), proteasome assembly (POMP), and ubiquitin recruitment/recognition (ADRM1, VCP) - were unanticipated. Interestingly, the deeply conserved NRF2 targets are enriched for genes encoding proteins found in exosomes (p=0.003), which are extracellular microvesicles that facilitate communication between cells. Therefore we hypothesized that NRF2 activation would result in increased exosome production. We treated cells with either 20µM of the NRF2 activator sulforaphane or vehicle and quantified the number of exosomes from the culture media using NanoSight-NTA. The sulforaphane treated cells produced significantly more exosomes than the vehicle-treated group (fold change 2.87±1.13, p=0.005) Additionally, we found that NRF2 target genes are regulated using either switch-like or graded regulatory strategies, and deeply conserved positive and negative feedback loops likely contribute to proper quantitative regulation of distinct NRF2 target genes. Combined, our findings highlight the importance of balance in the ancient NRF2 pathway, and indicate that NRF2-mediated regulation of redox homeostasis, metabolism, proteostasis, and cell non-autonomous responses to stress (exosomes) are ancient mechanisms that have always been central to this pathway.

2308 Annexins: An Early Cell Signaling Response to Environmental Toxicants Relevant to Tumorigenesis

B. L. Upham[2], I. Sovadinova[3], J.-S. Park[1], D. Whitten[2], C. Wilkerson[2] and P. Babica[4]. [1]*Laboratory Animal Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Korea, Republic of;* [2]*Pediatrics & Human Development, Michigan State University, East Lansing, MI;* [3]*Research Centre for Toxic Compounds in the Environment, Masaryk University, Brno, Czech Republic and* [4]*Research Centre for Toxic Compounds in the Environment and Department of Experimental Phycology, Masaryk University and Institute of Botany ASCR, East Lansing, Czech Republic;* [5]*RTSF Proteomic Core, Michigan State University, East Lansing, MI.*

Lower molecular weight polycyclic aromatic hydrocarbons (PAHs) with specific structural features are potent *in vitro* inhibitors of gap junctional intercellular communication (GJIC), and activators of arachidonic acid (AA) release and mitogen-activated protein kinases (MAPKs), which are cellular events linked with tumor promotion and other pathologies. We used advanced proteomics techniques (SILAC - Stable Isotopes Labeling with Amino Acids in Cell Culture, ZOOM® isoelectric fractionation, 1-DE, 2-DE and mass spectroscopic identification) to identify early upstream biochemical signaling events in a rat liver oval-like epithelial cell line (WB-F344). Proteins specifically affected within a 5 min exposure time to PAHs were annexins A1, A3 and A5, which disappeared from the plasma membrane, while annexins A2, A4, A6, A7 and A11 were unaffected. Immunostaining experiments on annexin A3 (AnxA3) indicated a translocation from the plasma membrane within 30 s of exposure followed by reintegration back into the plasma membrane after 60 min. Translocation of AnxA3 from the plasma membrane was effectively prevented by pre-treatment of the cells with PC-PLC inhibitor, D609. Knock-down of AnxA3 by siRNA did not prevent 1-MeA induced dysregulation of GJIC but did stimulate the 1-MeA induced release of AA. We hypothesize that annexins closely interact with phospholipases in the plasma membrane until translocated from the plasma membrane in response to a PAH, and the subsequent phospholipase-induced events then regulate the release of lipid derived second messengers. Support: NIEHS grant #R01 ES013268-01A2 to upham.

2309 Ligand-activated Aryl Hydrocarbon Receptor Inhibits Pancreatic Cancer Cell Invasion

U. Jin, Y. Chen and S. Safe. *VTPP, Texas A&M University, College Station, TX.*

The aryl hydrocarbon receptor (AhR) is a ligand-activated receptor that has been extensively characterized as a nuclear transcription factor that forms a transcriptionally-active nuclear complex with the AhR nuclear translocator (Arnt) protein. The AhR has multiple endogeneous and ligand-activated functions which include mediating the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds and also serving as a drug target for cancer chemotherapy. TCDD and the selective AhR modulator Omeprazole inhibited breast cancer cell invasion by nuclear AhR complex-mediated downregulation of pro-oncogenic CXCR4 expression. In contrast Omeprazole but not TCDD inhibited invasion of highly aggressive Panc1 and MiaPaCa2 pancreatic cancer cells and this response was AhR-dependent but independent of Arnt and was due to a non-genomic pathway. The unexpected cytosolic location of the AhR was confirmed in Panc1 cells and was unaffected by treatment with 20 nM of the nuclear export inhibitor Leptomycin B. The role of Omeprazole-induced kinases on inhibition of Panc1 cell invasion was investigated using a panel of kinase inhibitors and only SP600125, a JNK kinase inhibitor, significantly blocked invasion induced by Omeprazole. Potential candidate downstream genes were investigated by RNAseq after treatment of wild-type Panc1 cells and cells depleted the AhR by RNA interference with 200 µM Omeprazole. We observed that Omeprazole treatment modulated expression of 125 AhR-dependent genes associated with multiple pathways (11 pathways) and the role of key invasion/migration genes such as ID, SCYL3, PLAU, ULK1, FAT1, ARHGAP5, SOX9, FLOT2, ITGB1, NRP2, and ROBO1 are currently being investigated.

2310 A Putative Interaction of TSPO with NADPH Oxidase in Primary Microglia

M. K. Loth, S. R. Guariglia, J. Choi, D. B. Re and T. R. Guilarte. *Environmental Health Sciences, Columbia University Medical Center, New York, NY.*

Translocator protein 18 kDa (TSPO) is a glial protein that is extensively used as a biomarker of neuroinflammation. We have previously shown that TSPO ligands (TSPO-L) induce cellular functions in microglia consistent with an activated state, suggesting an important role of TSPO in the inflammatory response to brain injury. In particular, exposing microglia to TSPO-L (1-100 nM) induces ROS production that can be abrogated by NADPH oxidase (NOX) inhibitors suggesting an association between TSPO and NOX, a multi-subunit enzyme that is highly enriched in microglia and is a major source of ROS in the CNS. To further elucidate the relationship between TSPO and NOX, we determined the source of ROS production resulting from microglia exposure to TSPO-L. Intracellular and extracellular ROS production was inhibited by NOX inhibitors, but not by a mitochondria permeability transition pore inhibitor, indicating that the source of ROS production is from NOX and not from mitochondria. These findings were confirmed using the mitochondria specific ROS probe MitoSOX. To assess if TSPO and NOX are interacting, we performed co-immunoprecipitation experiments. The results indicate that gp91phox and p22phox co-immunoprecipitated with TSPO supporting a functional protein-protein interaction. Our findings provide novel insight on the potential function and subcellular localization of TSPO in microglia. To further explore the subcellular localization of TSPO, we performed triple-label immunofluorescent confocal imaging of TSPO with the mitochondrial protein VDAC and the principal NOX subunit gp91phox, both of which revealed significant colocalization. We are currently confirming our results using immuno-EM labeling. Together our findings suggest a novel interaction of TSPO with NOX in primary microglia with significant implications for a new understanding of TSPO glial cell biology and for the development of therapeutic strategies [supported by NIEHS grant ES007062 to TRG].

2311 Oxidative Stress Alters Developing Cell Fate via Transcriptional Networks

L. M. Walker, N. R. Sparks and N. I. zur Nieden. *Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA.*

Exposure to toxicants such as tobacco products can increase cellular levels of reactive oxygen species (ROS) and alter both transcriptional networks and cell fate. Previous work in mature tissues found increased nuclear activity of transcription factor FOXO3 under elevated ROS, triggering increased oxidative stress fighting response. Moreover, ROS activation of the cJun N-terminal kinase (JNK) signal transduction pathway encouraged posttranslational modification and nuclear translocation of FOXO3. In developing cells, however, the relationship between ROS manipulation of transcriptional networks and altered cell fate has yet to be clearly elucidated. This study examined the link between altered activity of upstream kinase regulators of FOXO3 and osteogenic cell fate under elevated ROS. Embryonic stem cells (ESCs) were employed as a model system to study development. ESCs were differentiated into osteoblasts and treated with tobacco product extracts to induce ROS. Decreased osteogenic calcification as well as decreased nuclear FOXO3 protein and FOXO3 interaction with cofactor β-catenin was observed in high ROS doses. Preliminary co-immunoprecipitation of β-catenin/FOXO3 in JNK knockout cells revealed decreased overall interaction of β-catenin and FOXO3 in high ROS cultures, suggesting the importance of posttranslational modification of FOXO3. Treatment of high ROS wild-type cultures with anisomycin, a JNK activator, produced a synergistic decrease in calcification, suggesting that JNK was already active in high ROS cultures. Subsequent western blot analysis revealed comparable JNK activation between low and high ROS cultures whereas AKT activation levels were found to be elevated in high ROS cultures. Taken together, our results suggest that aberrant upstream kinase signaling during oxidative stress may be responsible for altered β-catenin/FOXO3 interaction leading to subsequent ROS accumulation and inhibited osteogenesis.

2312 Bromochloroacetate-Dibromoacetate- and Bromodichloroacetate-Induced Toxicity to the J774A.1 Macrophages

E. Hassoun and X. Zeng. *Pharmacology and Experimental Therapeutics, University of Toledo, Toledo, OH.* Sponsor: E. Hassoun.

Bromochloroacetate (BCA), dibromoacetate (DBA), and bromodichloroacetate (BDCA) are by- products formed during the water chlorination process and are present in the drinking water at much lower levels than the fully chlorinated acetates under normal water conditions. The compounds were tested *in vitro* in the J744A.1 cells for 48h and were found to induce concentration-dependent increases in cell death and lactate dehydrogenase leakage at concentrations ranging between 0.1-0.8 mM of BCA and DBA, and 1-9 mM of BDCA. Increases in the production of cellular carbonyl proteins (CPs), and superoxide anion (SA) were observed at concentrations raging between 0.1- 0.4 mM of BCA and DBA, and 1-5 mM of BDCA. It is concluded that SA and CP production play significant roles in the induction of cellular death by the compounds, and that compounds concentrations associated with toxicities were significantly lower than the previously determined toxic concentrations of the fully chlorinated compounds in the same cell line.

2313 Investigation on the Effects of Oxidative Stress on Allergic Response Using Human Monocyte-Derived Cell

A. Suzuki, M. Mezaki and H. Itagaki. *Yokohama National University, Yokohama, Japan.*

Background/Purpose: Reactive oxygen species (ROS) is produced in cells by various stress. For example, heat shock, UV irradiation, and a variety of chemicals such as pollutants are proposed as the causes of ROS generation. Contact allergens such as dinitrochlorobenzene also generate intracellular ROS. Although hydrogen peroxide is not a contact sensitizer, it is known to upregulate the expression of CD54 and CD86, which are the predictive markers used in h-CLAT, an *in vitro* skin sensitization test using human monocyte cell line THP-1. This suggests that oxidative stress could be affecting a particular allergic response mechanism. In the present study, we used h-CLAT to investigate the effects on allergy-like responses that arise from the induction of oxidative stress on THP-1. We also examined the suppressive effect of antioxidant on the allergy-like response. Method: THP-1 was incubated in a serum-free or low-serum medium (FBS 0, 0.1, 1, & 5%) for 24 hrs. A plurality of antioxidants such as N-acetylcysteine was applied to quench intracellular ROS, and the effects were investigated. The h-CLAT protocol was followed in staining the cells with CD54 and CD86 antibodies, which were then analyzed using flow cytometry. Intracellular ROS was stained with H2DCFDA. Result/Discussion: The induction of oxidative stress on THP-1 per incubation in low-serum media upregulated the expression of CD54 and CD86. This result imitates the response of THP-1 to hydrogen peroxide. The application of antioxidants suppressed the expression of both CD54 and CD86 that had been induced by oxidative stress. These results suggest that oxidative stress might affect molecular expression in contact sensitization, and that this effect might be suppressed by antioxidants. Further analysis of the genes that are induced by ROS and related to the expression of CD54 and CD86 might provide more information to identify the molecular mechanism in which ROS is involved in the allergy-like response.

2314 Hypochlorite/Hypochlorous Acid-Mediated Oxidation of Methionyl Tripeptides

S. Babu[3], A. D. Logan[2], L. Tucker[3], K. Windham[3], R. M. Uppu[2] and M. O. Claville[3]. [1]*Department of Chemistry, Southern University and A&M College, Baton Rouge, LA;* [2]*Environmental Toxicology PhD Program, Southern University and A&M College, Baton Rouge, LA and* [3]*School of Science, Hampton University, Hampton, VA.*

Hypochlorite (¯OCl) and its conjugate acid, hypochlorous acid (HOCl; pKa ≈ 7.53) constitute a potent oxidant system elaborated by activated neutrophils and macrophages. Apart from being microbicidal, the ¯OCl/HOCl system causes tissue injury in a variety of pathological conditions. This type of tissue injury, often referred to as collateral damage, has been thought to be a consequence of the reactivity of ¯OCl/HOCl towards the side chains of certain amino acids, such as, cysteine, methionine, tyrosine, tryptophan and histidine, resulting in posttranslational modifications and/or inactivation of functional and structural proteins. Earlier experiments conducted in our laboratory showed that the ease of ¯OCl/HOCl-mediated oxidation of methionine (Met)-containing dipeptide depends on the position of Met and the amino acid position. In

this study, we synthesized four Met-containing tripeptides, namely, Ala-Gly-Met, Ala-Met-Gly, N-acetyl-Ala-Gly-Met and N-acetyl-Ala-Met-Gly, and studied the relative ease of oxidation by the ¯OCl/HOCl system. The peptides used in this study were deliberately acetylated at the N-termini in order to gain insight into the possible role of the amino group during the oxidation. The reactions were performed with 1 to 2 µmol of ¯OCl/HOCl in 2 mL of 0.1 M phosphate buffer, pH 7.0 that also contained 0.1 mM DTPA, while keeping the concentration of Met-peptide constant at 1 mM (total 2 µmol) . The reactions were monitored from 200 to 600 nm using a Varian Cary 50 Bio UV/Vis spectrophotometer. All the tripeptides showed strong absorption between wavelengths of 205 and 210 nm. ¯OCl/HOCl was consumed rapidly with the addition of Met-containing tripeptides which was evident from the instantaneous decrease in the absorption of the oxidant's peak at 290 nm. The Met-containing tripeptides were fully oxidized with as little as 50 mol% excess of ¯OCl/HOCl. It was observed that the oxidation of peptides occurs at concentrations close to those found in biological systems (i.e, 25-200 µM). We summarize Met peptides can act as chain-breaking antioxidants and have the ability to prevent the radical propagation initiated by ¯OCl/HOCl, thereby reducing its toxicity [Supported by NSF grants CHE-1230357 (CAREER) and HRD-1238838 (HBCU-UP ACE)].

2315 Long-Term Effects of Prenatal Exposure to Low Doses of Internal Radiation on the Hippocampal Function

M. Bellés[2], J. V. Vallvé[2], N. Serra[2], J. L. Domingo[2] and V. Linares[2]. [1]*School of Medicine, Universitat Rovira i Virgili, Reus, Spain and* [2]*Toxicology, Universitat Rovira i Virgili, Reus, Spain.*

Pregnant women are at risk of exposure to ionizing radiation (IR) resulting from necessary medical procedures, workplace exposure, diagnostic or therapeutic interventions, and even potential accidental cases in nuclear facilities. It has been reported that pre- or postnatal irradiation may cause perturbation of brain development, which might have serious consequences for adult brain structure and function. Radiation-induced cognitive dysfunctions are often manifested as deficits in hippocampal-dependent functions of learning and memory. However, the possible molecular mechanisms involved in hippocampal dysfunction after early exposure to IR, as well as the long-term effects in cognitive functions need to be clarified. In this study we investigated the hippocampal function in adult mice prenatally exposed to low doses of internal IR. C57Bl/6J mice were exposed subcutaneously to 137Cs at activities of 0, 500, 1000, 2000, 4000, and 8000 Bq/kg on gestation day 12 (GD12). Inflammatory cytokines markers (IL-1β, IL-6, IL-2) and expression of target genes (BDNF, p-53, HO-1) in the hippocampus were analyzed at 4 months of age. The results showed that the cytokine levels were higher in all internal irradiated mice compared with control animals. The expression of BDNF and HO-1 tended to increase in all exposed mice, but significantly at the lower 137Cs activity (500 Bq/kg). However, no differences between control and irradiated groups were noted in the p-53 expression values. These data suggest that low doses of IR can induce a complex response of reactive processes involving oxidative stress, neurogenesis and inflammation in hippocampal tissue. These alterations may be associated with long-term cognitive effects.

2316 Potential Roles of Diclofenac Acyl Glucuronide in the Pathomechanism of Intestinal Toxicity After High Dose Diclofenac

R. J. Scialis[2], I. L. Csanaky[1,3] and J. E. Manautou[2]. [1]*Department of Pediatrics, University of Kansas Medical Center, Kansas City, KS;* [2]*Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT and* [3]*Division of Clinical Pharmacology, Toxicology and Therapeutic Innovation, Children's Mercy Hospital & Clinics, Kansas City, MO.*

We previously reported that mice lacking the efflux transporter Mrp3 (Abcc3), which plays an important role in mediating intestinal disposition, had greater intestinal injury compared to wild-type mice following acute toxic diclofenac (DCF) administration. One potential explanation is that diclofenac acyl glucuronide (DCF-AG), a substrate for Mrp3, contributed to the injury. As both humans and mice express the uptake transporter organic anion transporting polypeptide (OATP)2B1 (SLCO2B1) in the intestine, OATP2B1 was characterized for DCF-AG uptake using stably transfected HEK-OATP2B1 cells. DCF-AG was transported by OATP2B1 with a Vmax and Km of 17.6 ± 1.5 pmol/min/mg and 14.3 ± 0.1 µM, respectively. DCF-AG was also found to be cytotoxic, more so than DCF itself, with cytotoxicity occurring within 3 hours of exposure. The potential for DCF-AG to produce reactive oxygen species (ROS) was also investigated. DCF-AG (1 mM) caused a 6-fold increase in ROS by 3 hours. Further investigations on oxidative stress quantified the ability of DCF-AG to inhibit superoxide dismutase (SOD). Whereas DCF increased SOD activity, DCF-AG produced 100% inhibition of SOD at 1 mM. Lastly, DCF-AG was screened for pharmacologic activity against COX-1 and COX-2 and was found to have IC50 values of 0.620 ± 0.105 µM and 2.91 ± 0.36 µM, respectively. Since COX inhibition can lead to intestinal ulceration, it is possible that DCF-AG, in addition to its ability to induce oxidative stress, can also contribute to enteropathy via COX inhibition. In summary, these studies demonstrate that DCF-AG can act as a direct contributor to cytotoxicity following DCF administration through synergistic pathways.

2317 Asbestos Induced Oxidative Stress in Lung Cells

L. Weng[4], C. Mesaros[4], N. W. Snyder[1] and I. Blair[4]. [1]*A.J. Drexel Autism Institute, Drexel University, Philadelphia, PA;* [2]*Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA;* [3]*Department of Systems Pharmacology & Translational Therapeutics, University of Pennsylvania, Philadelphia, PA and* [4]*Penn SRP Center, University of Pennsylvania, Philadelphia, PA.* Sponsor: T. Penning.

Pronounced asbestos exposure in the community of Ambler, PA has resulted in high prevalence of asbestos-related diseases including lung cancer and mesothelioma. Unfortunately, the pathogenic connections between asbestos exposure and the related diseases have not been well characterized. Various *in vivo* and *in vitro* studies suggest that oxidative stress induced by asbestos plays crucial role in developing asbestos-related diseases. The aim of this study is to determine the oxidative stress caused by asbestos from Ambler site and compare the effects with crocidolite, the well-characterized amphibole fiber. In mammalian cells, intracellular glutathione (GSH) provides one of the major defenses against oxidative stress by being oxidized to glutathione disulfide (GSSG). Consequently, the redox potential of 2GSH/GSSG indicates the cellular redox status. Herein, we quantified cellular GSH by stable isotope dilution liquid chromatography/multiple reaction monitoring mass spectrometry (LC-MRS-MS) following derivatization with 4-fluoro-7-sulfamoylbenzofurazan (ABD-F), which was an efficient method for stabilization and detection of GSH previously developed in our laboratory. Exposure to Ambler asbestos or crocidolite significantly decreased the intracellular GSH level, suggesting elevated oxidative stress induced by asbestos from either source. Furthermore, increased levels of 8-oxo-dG was observed in cells exposed to both asbestos fibers, suggesting DNA damage resulted from elevated oxidative stress due to asbestos exposure. This study illustrated that exposure to asbestos significantly raised the cellular oxidative stress. The difference in the level of DNA damage observed between Ambler and crocidolite asbestos suggested that the cellular response to asbestos exposure could be a consequence of each composition and that each composition may affect cellular pathways at different potencies.

2318 Dietary Antioxidants Counteract Ethanol-Induced Morphological Changes in Bones of Female Mice

A. Alund. *The University of Arkansas for Medical Sciences, Little Rock, AR.* Sponsor: M. Ronis.

Chronic alcohol consumption leads to an elevated risk of osteoporosis as well as increased fracture risk by decreasing bone mineral density through increasing bone resorption and decreasing bone formation. Our lab has shown that this is due, in part, to an increase in reactive oxygen species (ROS) produced by NADPH Oxidases (NOX). It was hypothesized that different dietary antioxidants, curcumin (120 mg/kg/d), N-acetyl cysteine (NAC) (1.2mg/kg/d), and vitamin E (α-tocopherol) (60mg/kg/d) would be able to attenuate the NOX derived ROS effects on bone due to chronic alcohol intake. In this study, female mice received a high fat (35%) Lieber DeCarli liquid diet supplemented with an antioxidant with or without EtOH at 30% of total calories for 8 weeks. MicroCT analysis showed a significant decrease in trabecular bone volume (BV/TV) and trabecular number (Tb.N) ($P<.05$), along with a significant increase in trabecular spacing (Tb.Sp) in the EtOH compared to PF ($P<.05$). In contrast, the EtOH+NAC and EtOH+α-tocopherol did not statistically differ from their respective PF controls. *Ex vivo* sections of tibias were stained for nitrotyrosine, an indicator of intracellular damage by ROS, and as expected tibias from mice fed EtOH had significantly more staining compared to PF controls. EtOH also significantly increased the size and number of marrow adipocytes per mm as well as mRNA expression of aP2, an adipocyte marker in bone. NAC was able to reduce the number of marrow adipocytes to PF levels while vitamin E and curcumin were not as effective. EtOH fed mice exhibited reduced bone length ($P<0.05$) and had a reduced number of proliferating chondrocytes within the growth plate. NAC and Vitamin E prevented this ($P<0.05$). These data show that alcohol's pathological effects on bone extend far beyond decreasing bone mass. Funded in part by R01 AA018282 (M.R) and CUMG (M.R.).

2319 Elevated Plasma Methionine Sulfoxide and Methionine in Non-Survivors of Acetaminophen Overdose

K. H. Liu[4], M. R. McGill[2], J. Chandler[4], S. Banton[4], K. Uppal[4], D. Walker[4], S. Curry[1], S. Li[4], H. Jaeschke[3] and D. Jones[4]. *[1]Banner Good Samaritan Medical Center, University of Arizona College of Medicine, Phoenix, AZ; [2]Department of Pathology and Immunology, Washington University at St. Louis Medical School, St. Louis, MO; [3]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS and [4]School of Medicine, Emory University, Atlanta, GA.*

Acetaminophen (APAP) overdose is the leading cause of acute liver failure in the United States, causing approximately 500 deaths annually. To better understand metabolic factors distinguishing survival from non-survival, we performed a discovery based metabolomics analysis of plasma from 10 survivors and 7 non-survivors of acetaminophen overdose with the goal of identifying metabolic differences that could discriminate the two groups. Results showed that from 17,172 mass spectral features analyzed with a stringent false discovery rate (q < 0.02), methionine sulfoxide (MetSO), methionine (Met), alanine, phenylalanine, and tyrosine were elevated in non-survivors compared to survivors. These results indicate severe negative nitrogen balance and/or decreased hepatic clearance of amino acids in non-survivors. Further analysis with a complementary chiral chromatography mass spectrometry method revealed MetSO exists primarily as Met(S)SO, indicating mechanisms responsible for stereospecific reduction of MetSO or stereospecific oxidation of Met could be important in mediating recovery from APAP overdose. Decreased stereospecific reduction of Met(S)SO could result from inhibition of methionine sulfoxide reductase A (MsrA). MsrA activity is partially dependent on the thioredoxin (Trx) system, and a recent study showed decreased thioredoxin reductase activity following APAP administration. Stereospecific production of Met(S)SO can be catalyzed through Met S-oxidase activity of MsrA, which can occur in Trx-compromised systems, or through flavin monooxygenase 3, an enzyme recently observed to be induced in a mouse model of APAP. Elevated plasma Met(S)SO may also reflect impaired hepatic clearance of Met, as Met levels were elevated 10-fold in non-survivors and 2.5-fold in survivors compared to control, and previous studies have observed increased Met(S)SO after Met loading in mice. Mechanisms involved in regulating free Met(S)SO concentrations following APAP overdose are not fully understood and require further study.

2320 Assessing Effects of Environmental Quinones on Mitochondrial Function in Human Airway Epithelial Cells Using Extracellular Flux Technology

K. S. Lavrich[1], P. A. Wages[1], D. Suarez[3], S. O. Simmons[3] and J. M. Samet[2]. *[1]Curriculum in Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]EPHD, NHEERL, US EPA, Chapel Hill, NC and [3]NCCT, US EPA, Research Triangle Park, NC.*

Ambient air pollution exposure is linked to increased mortality and morbidity in humans, primarily affecting the cardiopulmonary system. Oxidative stress mechanisms attributed to air pollutant-induced disease development are not well defined. Ambient air pollution is a complex mixture. Quinones are one component known to have toxicologic effects on humans. Quinones are released to the air primarily through fuel combustion. The most prevalent quinones in ambient air include 1,2-naphthoquinone (1,2-NQ), 1,4-naphthoquinone (1,4-NQ), 9,10-phenanthrenequinone (9,10-PQ), and 1,4-benzoquinone (BQ). Quinones have two primary mechanisms of toxicity - electrophilic addition and redox cycling, though different compounds vary their propensity for each mechanism. Previous work showed 1,2-NQ exposure increased ROS production and induced adaptive gene signaling pathways in human airway epithelial cells. We sought to identify key mechanisms of ROS production by environmental quinones. Since mitochondria are the major source of cellular ROS, we characterized the effects of quinone exposure on mitochondrial function in human airway epithelial cells using extracellular flux technology. We measured oxygen consumption rate (OCR), a read-out of mitochondrial function, using the XFe Analyzer (Seahorse Bioscience) in the immortalized human bronchial epithelial cell line BEAS-2B. Environmentally-relevant doses of 1,2-NQ, 1,4-NQ, and 9,10-PQ, quinones with redox cycling capabilities, significantly increased OCR within 2.5 minutes of exposure. In contrast, the non-redox cycling BQ does not affect OCR. The OCR rise appears to be independent of mitochondrial electron transfer, while interventions that decrease NADPH levels strongly blunt the OCR increase induced by redox cycling quinones. In a permeabilized cell mitochondrial preparation, 1,2-NQ and 1,4-NQ appear to inhibit mitochondrial function. These results suggest cytosolic redox cycling is the primary mechanism for 1,2-NQ-induced OCR elevation leading to ROS production in the human airway following the inhalation of environmental quinones. This Abstract of a Proposed Presentation Does Not Necessarily Reflect US EPA Policy.

2321 Hydroxylated Non-Steroidal Anti-Inflammatory Drugs as a Source of Cytotoxic Free Radical Metabolites

A. G. Morgan, K. Michail, S. Khan and S. G. Arno. *University of Alberta, Edmonton, AB, Canada.*

Idiosyncratic adverse drug reactions (IADRs) represent a major concern for patients, pharmaceutical companies, and regulatory bodies. IADRs have been reported to occur with widely used drugs, for example, drug induced liver injury and hemotoxicity have been associated with diclofenac and indomethacin. Although the toxicity mechanisms are not fully understood, it is largely believed that reactive metabolites are implicated in serious IADRs through sequential toxicological events. This study particularly investigates the potential of myeloperoxidase (MPO)-mediated bioactivation, reactivity, and *in vitro* toxicity of diclofenac, indomethacin and naproxen in comparison with their major hydroxylated hepatic metabolites, 4'-hydroxydiclofenac (4'-OHD), 5-hydroxydiclofenac (5-OHD), O-desmethyl-N-deschlorobenzoyl indomethacin (DMBI), O-desmthylindomethacin (DMI) and O-desmethylnaproxen (ODN). For determining the reactivity of the metabolites produced from MPO, we used kinetic UV-visible spectroscopy to determine ascorbate cooxidation rates; also, we detected glutathionyl radical (GS.) induced by NSAID free radical metabolites using electron spin resonance with DMPO (5,5-Dimethyl-1-Pyrroline-N-Oxide) spin trapping. For *in vitro* toxicity, we used trypan blue exclusion in HL-60 cells, which contain high amounts of MPO. Also, a cytofluorometric glutathione assay was performed in HL-60 cells, and changes in HL-60 mitochondrial membrane potential were studied using JC-1 fluorescence. We found that the only compounds bioactivated by MPO that cooxidized ascorbate were 4'-OHD, ODN and DMBI. In addition, 4'OHD and ODN were oxidized by MPO to reactive species that oxidized GSH, forming GS. radical, which was not formed with DMBI reactive species, suggesting that the latter is a non-free radical reactive species. Even though all the three species shared the ability to oxidize ascorbate, deplete cellular GSH and to decrease mitochondrial membrane polarization, only a 4'OHD-derived free radical species was able to cause significant cytotoxicity. In conclusion, metabolism of NSAIDs' hydroxylated metabolites, namely 4'-OHD,ODN and DMBI by MPO results in deleterious species, that are variable in nature and reactivity, and could potentially be implicated in the development of IADRs associated with the use of their parent drugs.

2322 Nuclear Factor-κB1/RelA Mediates Inflammation in Human Lung Epithelial Cells at Atmospheric Oxygen Levels

L. Jagannathan[2], C. C. Jose[2], A. Arita[2], T. Kluz[2], H. Sun[2], X. Zhang[2], Y. Yao[2], A. V. Kartashov[1], A. Barski[1], M. Costa[2] and S. Cuddapah[2]. *[1]Division of Allergy & Immunology and Human Genetics, Cincinnati Children's Hospital Medical Center and Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, OH and [2]Environmental Medicine, NYU Langone Medical center, Tuxedo, NY.*

Oxygen levels range from 2-9% *in vivo*. Atmospheric O2 levels (21%) induce oxidative stress due to generation of reactive oxygen species (ROS) in primary cells cultured at higher than physiological levels is implicated in a multitude of deleterious effects including DNA damage, genomic instability and senescence. However, the mechanistic basis of the deleterious effects of higher O2 levels is not fully understood. Moreover, immortalized cells including cancer cell lines, which evade cellular senescence are normally cultured at 21% O2 and the effects of higher O2 on these cells are understudied. Here we addressed this problem by culturing immortalized human bronchial epithelial (BEAS-2B) cells at ambient atmospheric, 21% O2 and lower, 10% O2. Our results show increased inflammatory response at 21% O2 but not at 10% O2. We found higher RelA binding at the NF-κB1/RelA target gene promoters as well as upregulation of several pro-inflammatory cytokines in cells cultured at 21% O2. RelA knockdown prevented the upregulation of the pro-inflammatory cytokines at 21% O2, suggesting NF-κB1/RelA as a major mediator of inflammatory response in cells cultured at 21% O2. Interestingly, unlike the 21% O2 cultured cells, exposure of 10% O2 cultured cells to H2O2 did not elicit inflammatory response, suggesting increased ability to tolerate oxidative stress in cells cultured at lower O2 levels.

2323 Does a Fish Model Matter? Comparative Toxicology of Oxidative Stress Toxicity in Zebrafish and Fathead Minnows

J. Corrales[4], L. Kristofco[4], B. Steele[4], S. Williams[4], M. Mills[3], E. Gallagher[3], T. Kavanagh[3], L. Shen[1], F. Melnikov[1], J. Kostal[2], A. Voutchkova-Kostal[2], P. Anastas[1] and B. Brooks[4]. *[1]Center for Green Chemistry & Green Engineering, Yale University, New Haven, CT; [2]Department of Chemistry, The George Washington University, Washington, DC; [3]Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and [4]Environmental Science, Baylor University, Waco, TX.* Sponsor: B. Brooks.

Over 85% of the approved commercial chemicals in the US lack experimental health and safety data. The fourth Principle of Green Chemistry states that "chemical products should be designed to preserve efficacy of function while minimizing toxicity". To accomplish this goal and to identify less hazardous alternative chemicals, alternative toxicology testing approaches, including fish models, provide novel insight to toxicity profiles. In this project, we selected the Nrf2 pathway to initially identify compounds that elicit oxidative stress responses. Nrf2 is regarded as a main regulator of oxidative stress response genes. We employed *in vivo* toxicological data in two model species, zebrafish and fathead minnow, resulting in a unique comparative toxicity approach. The goal of the study was to define relationships between traditional endpoints and oxidative stress responses following exposure. Such information is necessary prior to derivation of computational models that associate oxidative stress parameters with chemical attributes. Following OECD FET and EPA WET guidelines zebrafish embryos and fathead minnow larvae were exposed for 96 h to bisphenol A, cumene hydroperoxide, dinoseb, hydroquinone, indene, perfluorooctanoic acid, R-carvone, and tert-butyl hydroperoxide. Range-finding tests were conducted to determine 96 h LC50 values, and fish were then exposed to LC50, 40% LC50, 20% LC50, 10% LC50, 5% LC50, solvent control, and control. Mortality was recorded daily. At 96 h, larvae were collected in triplicate to measure lipid peroxidation, total glutathione, DNA damage, and mRNA expression of catalase, gstp, gclc, superoxide dismutase, and nrf2. Differential sensitivities were observed between fish models, chemicals, and response variables examined. Results indicated that oxidative stress responses do not consistently translate across fish models, and selection of model organism should be further examined prior to mechanistic alternative toxicology studies with industrial chemicals.

2324 Towards Less Hazardous Industrial Chemicals: Comparative Behavioral Toxicology of Two Common Fish Models

B. Steele[4], L. Kristofco[4], J. Corrales[4], M. Mills[2], S. Williams[4], F. Melnikov[3], L. Shen[3], J. Kostal[1], A. Voutchkova[1], P. Anastas[3], T. Kavanagh[2], E. Gallagher[2] and B. Brooks[4]. *[1]George Washington University, Washington, DC; [2]University of Washington, Seattle, WA; [3]Yale University, New Haven, CT and [4]Environmental Science, Baylor University, Waco, TX.* Sponsor: B. Brooks.

Behavioral responses inform toxicology studies by rapidly and sensitively detecting molecular initiation events that propagate to physiological changes in individuals. Behavioral thresholds for a wide range of contaminants identify bioactivity and routinely are observed at lower levels than those eliciting mortality and other standardized adverse outcomes. We employed the zebrafish and fathead minnow models to define toxicant induced swimming activity alterations during interchanging photoperiods. We specifically compared behavioral response patterns among eight industrial compounds (bisphenol A, cumene hydroperoxide, dinoseb, hydroquinone, indene, perfluorooctanoic acid (PFOA), R-carvone, tert-butyl hydroperoxide) in the US EPA ToxCast program that are known to cause oxidative stress. Following OECD FET and EPA WET experimental guidelines, zebrafish embryos and fathead minnow larvae were exposed for 96 h to each compound, then photomotor responses observed using a digital behavioral analysis system (ViewPoint). Behavioral observations occurred for 50 minutes with altering light/dark 10 minute photoperiods. Zebrafish exposed to hydroquinone exhibited increased swimming activity ($p<0.05$) at higher speeds during dark photoperiods, and fathead minnows swam greater distances ($p<0 .05$) at higher speeds during dark periods when exposed to PFOA. All of the other compounds to sublethally affect larval swimming behavior decreased ($p <0.05$) activity under light and/or dark conditions. Chemical differences in behavioral responses to light and dark conditions among these 8 compounds may have resulted from diverse mechanisms of action. The approach initially explored in the present study is presently being augmented with additional pharmaceutical, pesticide and industrial chemical data to support development of novel computational models for fish behavior, and ultimately the derivation of sustainable molecular design guidelines for less hazardous chemicals.

2325 Multiorgan Oxidative Damage Caused by Subchronic Exposure to Benzene or Hydroquinone

R. Ruiz-Ramos[2], H. García-Hernández[2], M. L. Betancourt-Aldazaba[2], N. López-Amador[2], A. L. Calderón-Garcidueñas[2], W. E. Becerra-Romero[2], H. Vivanco-Cid[2] and S. M. Waliszewski[2]. *[1]Instituto de Investigaciones Médico Biológicas, Universidad Veracruzana, Veracruz, Mexico and [2]Instituto de Medicina Forense, Universidad Veracruzana, Veracruz, Mexico.*

Benzene (BZ) is a low molecular weight hydrocarbon, which is a ubiquitous environmental pollutant in highly industrialized cities. BZ is a class I carcinogen and its oxidation to reactive intermediates is a prerequisite of their toxicity. Metabolites generated during exposure include hydroquinone, which is highly toxic, but it is also known that exposure to BZ produce reactive oxygen species (ROS), which are capable of causing cell damage in parallel. However, it is not fully known the oxidative damage caused by BZ or HQ in multiple organs, following exposures for long periods. Quantification of lipid peroxidation is essential for assessing the role of oxidative injury in pathophysiological disorders. Therefore, we studied the oxidative damage caused by exposure to BZ or HQ by evaluating lipid peroxidation (LPO) in multiple organs of rats exposed subchronically. Wistar rats treated intragastrically with BZ or HQ during 45 days showed lipoperoxidation levels up to 3.5 fold compared to the control in liver, lungs, brain, kidney, heart and spleen. However, simultaneous addition of alpha-tocopherol, only reduced generation of oxidative damage mediated by BZ in heart, while HQ mediated damage, was reduced in all organs analyzed. These observations are consistent with changes in body weight of rats exposed to both HQ or BZ, and the protective effects of alpha-tocopherol. These results suggest that exposure to BZ or BQ for prolonged periods can cause oxidative damage that can lead to pathophysiological disorders in addition to the known carcinogenic effects

2326 Redox Proteomics and Metabolomics Reveal Distinct Effects of Thiocyanate Switching of Oxidants in Human Lung Epithelia

J. D. Chandler, V. Tran, M. L. Orr, K. Liu, Y.-M. Go and D. P. Jones. *Emory University, Atlanta, GA.*

Inflammation results in production of H2O2, which can be converted to hypochlorous (HOCl) and hypobromous (HOBr) acids by myeloperoxidase *in vivo*. Thiocyanate converts these to hypothiocyanous acid (HOSCN), switching oxidant exposure. The oxidants differ in reactivity and selectivity in spite of their superficial similarities; e.g., methionine (Met) is rapidly oxidized by HOCl and HOBr, but HOSCN reacts preferentially with cysteine (Cys). Redox-active proteins also discriminate among these oxidants, e.g. thioredoxin reductase metabolism of HOSCN but not HOCl. Therefore, we used molecular methods and mass spectrometry (MS) to quantify oxidant-specific redox small molecule, redox proteome and metabolome effects upon exposure of BEAS-2B lung epithelial cells to equal doses of each. Intracellular glutathione (GSH) oxidation was only appreciable with HOSCN and H2O2 at 2 and 6 h, but extracellular Cys and GSH were oxidized most rapidly by HOSCN. However, mitochondrial peroxiredoxin 3 was only oxidized by H2O2. By 24 h, HOCl, HOBr and H2O2 caused cytotoxicity (up to 75% LDH release) but HOSCN did not. Using MS-based redox proteomics and metabolomics platforms, we observed that HOSCN decreased oxidation of Cys biosynthesis enzymes (methionine adenosyltransferase C104, adenosylhomocysteinase C195) and metabolic precursors (S-adenosyl Met and glutamate) of GSH, consistent with 4.0- and 1.4-fold increases in Cys and GSH abundance. HOSCN also oxidized many protein Cys, including phosphatases (PP2A, C13; PP2C, C84), cytoskeletal proteins (profilin, C128; cofilin, C139) and glycolytic enzymes (ALDOA, C232; GPI, C443), and maximally perturbed 581 metabolites. In contrast, HOCl and HOBr caused more rapid disruption of metabolites and maximally perturbed 1068 and 995 (respectively) after 24 h. HOCl and HOBr alone potently abolished intracellular Met, increasing Met sulfoxide (MetO) abundance >1000-fold. Targeted MS2 with chiral separation revealed Met(S)O:Met(R)O stereoisomer ratio was oxidant-dependent, with HOCl and HOBr favoring higher relative Met(S)O (ratio of 1.47 vs. 1.17 for control), implicating altered MetO reductase activity in the context of the different oxidants. These data show that oxidants with superficial similarities greatly differ in redox proteomic and metabolomic targets in lung epithelia, associated with different toxic potential.

2327 Biopterin Oxidation and NOS Uncoupling: Regulators of Macrophage Function in Acute Lung Injury

T. Golden[2], M. Crabtree[1] and A. Gow[2]. [1]*Oxford University, Oxford, United Kingdom and* [2]*Rutgers University, Piscataway, NJ.*

Inducible nitric oxide synthase (iNOS) has been implicated in intratracheal-bleomycin (ITB) mediated acute lung injury (ALI). iNOS expression is increased in pulmonary epithelial cells and classically activated (M1) macrophages during the inflammatory phase of ITB-mediated ALI. ITB causes ALI via production of reactive species causing epithelial cell injury. Antioxidant therapeutic approaches have reduced ITB-induced inflammation. Tetrahydrobiopterin (BH4) is an essential cofactor for NOS production of NO that is readily oxidized. BH4 levels are increased during inflammation. Oxidized biopterins cannot function as cofactors, which leads to NOS uncoupling and further oxidative stress. This represents a mechanism of endothelial dysfunction but has not been directly investigated in the context of iNOS function. It is our hypothesis that during ALI BH4 is oxidized leading to further oxidative stress from uncoupled iNOS. C57BL/6 mice were treated with ITB (3U/kg) or saline. Cells were harvested from lungs by bronchoalveolar lavage and phenotypically assessed by flow cytometry. NOS products (NOx) were measured in the lining fluid. Tissues were collected for histology and biopterin measurement via HPLC. Inflammatory and resolution stages of ALI were studied 8 and 15 days post ITB respectively. 8 days post ITB, pulmonary inflammation was evident by histology and macrophage polarization favoured classical activation (M1). Despite M1 polarization, nitrite and nitrate levels were not increased. Total and oxidized biopterins were increased, but BH4 levels were not changed. The lack of reduced BH4 increases suggests iNOS uncoupling and a failure to produce nitric oxide. To examine the role of iNOS specifically in the lung, NOS2-/- mice were adoptively transferred with NOS2 competent bone marrow cells. Pulmonary inflammation was not altered by loss of iNOS in the lung, however lung macrophages failed to adopt a M1 phenotype at 8 days. Biopterin and NOx levels were not altered by loss of iNOS at 8 days. At 15 days post ITB, chimeric mice had increased inflammation and more M1 polarization compared to wild type. Oxidized biopterin levels in chimeric mice were elevated at 8 days post ITB but recovered to control levels at day 15. These preliminary data suggest that iNOS can be uncoupled by BH4 oxidation and that this may have significant effects on macrophage phenotypic differentitiation. GM108463, HL086621, ES005022, SFRBM

2328 Inhibition of S-Nitrosoglutathione Reductase (GSNOR) Reduces Lung Inflammation

S. Taylor, G. Voronin, D. Fett, T. Golden, D. Botehlo and A. Gow. *Pharmacology-Toxicology, Rutgers University, Piscataway, NJ.*

Under inflammatory conditions there is increased inducible Nitric Oxide Synthetase (iNOS) expression. iNOS generates NO· which can react with the thiols of small peptides/proteins to produce S-nitrosothiols (SNOs), leading to modification of cellular function. One such target is glutathione (GSHGSNO) and through transnitrosylation, is capable of further SNO formation. GSNO levels are regulated both by their formation and degradative mechanisms such as GSNOR. In this study we examined how inhibition of GSNOR altered acute pulmonary injury as a result of intratracheal bleomycin (ITB) administration, a model known to be modulated by iNOS function. In the absence of iNOS function, acute injury is reduced but long-term fibrosis is exacerbated. A number of intracellular targets involved in the inflammatory process are inhibited by SNO formation, such as NF-κB, while protective mechanisms, such as KEAP-1, are activated. Thus, we hypothesized that inhibition of GSNOR would decrease inflammation and reduce fibrosis in ITB through increased intracellular SNO formation. 8-12 week old, male C57BL6/J mice were administered 3 U/kg ITB or saline. Mice received the GSNOR inhibitor N6022 (or saline) daily by i.p. injection from days 0-8 (peak inflammation) or every 48hrs from days 8-21 (fibrotic endpoint). N6022 treated animals that received ITB had a reduced median inflammatory score compared to their saline controls ($p<0.05$ by Wilcoxon Rank Sum Test), indicating that increased SNO reduced the injury response. RT-qPCR analysis of cells from broncho-alveolar lavage fluid revealed altered inflammatory signaling. N6022 administration reduced expression of inflammatory genes with the exception of ARG1, which remained unchanged compared to saline treatment. However, analysis of lung function, histology, and additional endpoints revealed no reduction in fibrosis as a result of N6022 administration. Taken together, it appears that increased GSNO reduces inflammation without affecting fibrosis in the ITB model. This work is supported by NIH sponsored T32ES007148 and HL086621 grants.

2329 Air Pollutant 9,10-Phenanthrenequinone-Induced Endothelial Cell Dysfunction: Roles of ROS, NF-kB and NQO1

Z. Jia[3], P. Nallasamy[3], H. Zhu[1], W. Wu[4], S. Halley[3], M. A. Trush[2], K. Traore[1] and Y. R. Li[1]. [1]*Campbell University School of Osteopathic Medicine, Buies Creek, NC;* [2]*Johns Hopkins University Bloomberg School of Publi, Baltimore, MD;* [3]*Biology, University of North Carolina at Greensboro, Greensboro, NC and* [4]*School of Public Health, Xinxiang Medical University, Xinxiang, Henan Province, China.*

Atherosclerosis is the leading cause of morbidity and mortality in the United States and worldwide. Among the various risk factors of atherosclerosis, environmental pollution, particularly airborne particulate matter has recently been recognized as an important contributor to human atherosclerosis, but, the mechanism(s) remains to be elucidated. It has been shown that 9,10-phenanthraquinone (9,10-PNQ) alone compromises about 6% of the total organic extract of diesel exhaust particles. This study for the first time demonstrated that 9,10-PNQ at concentrations of 0.1, 0.3 and 1 micromolar significantly increased expression of monocyte chemoattractant protein-1, E-selectin, and interleukin -8 and intercellular adhesion molecule-1 in human aortic endothelial cells (HAECs). 9,10-PNQ also caused adhesion of monocytes to endothelial cells, a key event in the pathogenesis of atherosclerosis. Further, 9,10-PNQ increased the levels of reactive oxygen species (ROS) and induced nuclear factor-kappa B (NF-kB) transcriptional activity in endothelial cells. ES936 and dicumarol are inhibitors for NAD(P)H:quinone oxidoreductase 1 (NQO1) and were found to significantly block 9,10-PNQ-meditated ROS formation suggesting the crucial contribution of NQO1 to 9,10-PNQ-meditated redox activation in HAECs. The results from this study, taken together, may contribute towards the assessment of cardiovascular risk of human exposure to 9,10-PNQ and signifies the importance of ROS, NQO1 and NF-kB pathways.

2330 Cardiotoxic Effects of 1,1-Difluoroethane Due to Oxidative Stress and Electrolyte Changes

K. Joshi, C. A. Lau-Cam, M. Barletta and J. Wurpel. *Pharmaceutical Sciences (Toxicology), St. John's University, Queens, NY.*

Inhalant abuse is the intentional inhalation of chemical vapors to attain euphoric effect. Many common household products are abused by inhalation and one is 1, 1-Difluoroethane (DFE). DFE is a halogenated hydrocarbon used in refrigeration, dust-off spray and airbrush painting. Although many human DFE cases have been studied, the etiology and mechanism of sudden death is still unknown. Our earlier electrocardiographic (EKG) monitoring studies showed DFE treated animals died from severe arrhythmias. This research also monitored effects of multiple inhaled doses of DFE on the EKG. In this study an animal model was used to simulate the human conditions of DFE inhalation abuse that result in sudden death Current research targets mechanistic studies involving electrolyte changes and oxidative stress markers after DFE administration *in vivo*. To investigate these changes, Sprague Dawley rats (n=6) were exposed to 30 seconds of 20 L/min of DFE in multiple doses. Isoflurane acted as a control. Two additional groups, Epinephrine and Epinephrine + DFE were included which simulate clinical condition of DFE abuse. Following DFE administration, heart and blood were collected. Sodium, potassium, calcium and magnesium levels were measured followed by Lactate dehydrogenase (LDH) and Troponin I (cTnI) levels. Oxidative stress markers; Malondildehyde (MDA) and Catalase (CAT) were evaluated in all animal groups. Electrolyte levels showed a significant rise in plasma potassium and magnesium levels while sodium and calcium levels were not altered. In addition, LDH and cTnI levels in DFE and epinephrine + DFE administered rats were significantly elevated as compared to control. Oxidative stress makers MDA and CAT were elevated significantly in treatment groups. Further to check protective effects of anti-arrhythmic drugs, EKG was recorded after Propranolol and Metoprolol pretreatment followed by DFE administration. Total number of arrhythmias decreased and time for onset of death increased. These results support earlier cardiotoxic EKG effects indicating that DFE results in fatal arrhythmias. Further, histology experiments will be performed to confirm changes in oxidative stress markers.

2331 Environmentally Persistent Free Radicals Increase Systolic Blood Pressure and Block Compensatory Responses to Cardiac Stress in Rats with Ischemic Heart Disease

B. R. Burn[2,3], H. Xia[2,3] and K. J. Varner[2,3]. [1]*Cardiovascular Center of Excellence, LSU HSC, New Orleans, LA;* [2]*Pharmacology, LSU HSC, New Orleans, LA and* [3]*Superfund Research Center, LSU, Baton Rouge, LA.*

Increases in particulate matter (PM) are linked to increased mortality from cardiac ischemia. PM contains environmentally-persistent free radicals (EPFRs) that form when halogenated hydrocarbons chemisorb to the surface of transition metal oxide-coated particles. EPFRs are capable of sustained redox cycling in the environment. We showed that EPFRs exacerbate left ventricular (LV) dysfunction produced by ischemia-reperfusion injury. Here we tested the hypothesis that inhalational exposure to EPFRs would degrade LV performance in rats with preexisting ischemic disease. Myocardial infarction (MI), or Sham MI were performed 3 weeks before exposure to EPFRs or vehicle. Radio telemetry continuously recorded arterial pressure (AP) and heart rate (HR) before and after exposure. Pressure-volume catheters were used to assess LV function. Exposure to EPFRs (DCB230; 460 µg max/day) or vehicle 60 min/day, 5 days/week for 3 weeks was by nose-only inhalation. Before exposures each day, resting AP and HR were similar in the MI and Sham MI groups. AP and HR were not significantly different in sham rats exposed to DCB230 or vehicle. However, after 2 weeks of exposure, mean- and systolic AP were significantly greater in MI+DCB230 rats than in MI+vehicle rats. HR and diastolic AP were the same in both MI groups. MI evoked contractile compensation at baseline to maintain LV function. During dobutamine infusion, MI+DCB230 rats failed to increase preload recruitable stroke work (PRSW), stroke volume or stroke work indicating reduced contractile reserve. PRSW responses following β-blockade suggest alterations in sympathetic input in the MI+DCB230 group. In conclusion, EPFRs appear to increase systolic AP and reduce compensatory mechanisms in the ischemic heart which, may help to explain the correlations between PM2.5 and ischemic heart-related mortality.
Support P42ES013648; P30GM106392

2332 Triclosan Is a Proton Ionophore Mitochondrial Uncoupler in Living Cells Including Primary Human Keratinocytes: Effects on Reactive Oxygen Species Production and on Mitochondrial Morphology Using Super-Resolution Microscopy

L. M. Weatherly[3], A. J. Nelson[3], J. Shim[3], A. Riitano[3], H. N. Hashmi[3], R. Sher[3], S. T. Hess[3] and J. A. Gosse[3]. [1]*Department of Molecular and Biomedical Sciences, University of Maine, Orono, ME;* [2]*Department of Physics and Astronomy, University of Maine, Orono, ME and* [3]*Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME.*

Triclosan (TCS) is an antimicrobial used so ubiquitously that seventy-five percent of the U.S. population is likely exposed to TCS via consumer goods and personal care products. TCS is readily absorbed into human skin and has been found in urine and in the plasma and milk from nursing mothers. We previously showed that non-cytotoxic, µM levels of TCS inhibit both adenosine triphosphate (ATP) and oxygen consumption rate (OCR), from both rat (RBL-2H3) and human (HMC-1.2) mast cells. This effect is due to TCS's proton ionophore structure. Here, we show that TCS disrupts ATP production in NIH-3T3 mouse fibroblast cells cultured in glucose-free, galactose-containing media, with an EC50 of 4.8-7.4 µM (95% CI), without causing cytotoxicity. The same experiments were performed with primary human keratinocytes, yielding an even more pronounced decrease in ATP with an EC50 of 3.0-4.1 µM (95% CI) and no cytotoxicity. The reduction in ATP with no change in plasma membrane integrity in multiple cell types, including primary human cells, indicates that TCS is a mitochondrial toxicant. Known mitochondrial uncouplers have been shown to disrupt mitochondrial morphology. We have successfully imaged mitochondrial ultrastructure using fluorescence photoactivation localization microscopy (FPALM) with the outer mitochondrial membrane marker TOM20-dendra2 and have assessed the effects of TCS on this structure. TCS also increases reactive oxygen species (ROS) production in RBL-2H3 cells, in contrast to the effects of the known uncoupler CCCP, which decreases ROS production. These data show that TCS disrupts mitochondrial and cellular functioning in diverse cell types.

2333 Effects of Vitamin E Succinate on the Metastatic Prostate Cancer Cells

B. M. Rezk[1], M. Khanna[2], I. Urka[1], M. E. Khedr[2], R. Shaw[1], Z. Y. Abd Elmageed[2], S. C. Sikka[2] and A. B. Abdel-Mageed[2]. [1]*Natural Sciences, Southern University at New Orleans, New Orleans, LA and* [2]*Urology, Tulane University, School of Medicine, New Orleans, LA.*

Treatment of Prostate cancer with radiation and chemotherapeutic agents has been implicated in the induction of collateral damage to the normal tissues and increased risk of osteoporosis, cardiovascular disease, and diabetes. Thus, the strategy to use of such therapy seems to be an undesirable development and a search for agents that would either prevent prostate cancer or cure it without severe side effects is an essential demand. Because of the oxidizability of vitamin E (VE), supplementation with VE often occurs by adding more stable VE esters to VE formulations. Vitamin E succinate (VES) has been identified as a potent and a highly selective anticancer agent. This study has been designed to investigate the effects of VES on highly metastatic C4-2B and PC3 prostate cancer cell lines and compared these effects to RWPE-1 prostate normal cells. Our results showed that VES induced significant apoptotic effects on C4-2B and PC3 prostate cancer cell lines. Co-treatment with bicalutamide had neither synergistic nor additive effects in the androgen sensitive C4-2B prostate cancer cells. Importantly, low concentrations of VES enhanced the growth rate of prostate normal cell, RWPE1. The same findings have been shown with bicalutamide. Furthermore, VES did not alter total GSH and GSSG contents in RWPE-1 normal cells. VES induced pro-apoptotic effects and increased mitochondrial superoxide anion production in C4-2B and PC3 prostate cancer cell lines. These apoptotic activities were accompanied with reduction of TGSH contents in C4-2B and increased total GSH and GSSG levels in PC3 cells. Antibody array and RT-PCR results showed that VES has differential reduction effects on the expression of anti-apoptotic proteins HSP60, HSP70, SVV,XIAP and elevation of expression of a apoptotic protein Smac/DIABLO. In conclusions, our results showed that VES has remarkable and promising selective anticancer effects on the highly bone metastatic prostate cancer cell lines.

2334 Down-Regulation of Myeloid Cell Leukemia 1 (MCL-1) Protein in the Striatum Following Treatment with 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)

E. Lu, S. Sarkar, J. Raymick, M. G. Paule and Q. Gu. *Division of Neurotoxicology, US FDA National Center for Toxicological Research, Jefferson, AR.*

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a well-known neurotoxicant that can selectively destroy dopaminergic neurons in the brain and MPTP-treated animals are often used as models for studying molecular mechanisms associated with Parkinson's disease. While apoptosis has been suggested as a possible mechanism of MPTP-induced cell death and several apoptosis-associated proteins have been implicated in MPTP-models, relevant information is missing regarding the possible involvement of Mcl-1 (myeloid cell leukemia 1), which is an important member of the Bcl-2 family and is regarded as a highly regulated controller of cell death and survival. In the present study, an acute MPTP treatment regimen was utilized with male C57BL/6 mice (i.e. 10 mg/kg i.p. injections, 4 times with 2 hour intervals) and several protein markers were examined in MPTP and control animals 24-hours after the initial injection. Immunocytochemical labeling of tyrosine hydroxylase and dopamine transporters in the striatum indicated dramatic decrease in dopaminergic terminals. FluoroJade-C labeling also confirmed neuronal death in the MPTP-treated animals. Western-blot analysis further suggested a down-regulation of Mcl-1 protein levels in the striatum of MPTP-treated animals. Since Mcl-1 plays an inhibitory role in controlling BAX function, these results suggest that MPTP-induced cell death may be mediated by down-regulation of Mcl-1 and, consequently, activation of BAX proteins which form pores in the mitochondrial membrane and permit the release of cytochrome c from mitochondria into cytoplasm, which in turn activates down-stream caspases and other apoptosis processes.
Supported by US FDA/NCTR Protocols E0747701 and E0751201.

2335 Erk ½ Activation Is Evident in Activated Microglia From the Striatum and Substantia Nigra of MPTP-Induced Mouse Models of PD

S. Sarkar[1], E. Lu[1], J. Raymick[1], L. S. Schmued[1], J. Hanig[2], M. G. Paule[1] and Q. Gu[1]. *[1]Neurotoxicology, National Center for Toxicological Research, Jefferson, AR and [2]Office of Testing & Research, CDER/US FDA, White Oak, MD.*

Parkinson's disease (PD) is a debilitating, age-related disorder characterized by selective degeneration of dopaminergic neurons in the midbrain substantia nigra (SNc). Dopaminergic neurons originating here project to the striatum. Although studies have suggested that ERK ½ in the brain is activated after MPTP exposure, no study has yet been done to demonstrate whether such activation takes place in neurons or in glia. In the current study, we have utilized both an acute and a repeat dose mouse model of PD using the neurotoxicant MPTP as the causative agent. Immunohistochemical studies using phospho ERK ½ antibody suggest that ERK ½ activation takes place in both the striatum and SNc in both animal models. Moreover, double immunolabeling studies using phospho ERK ½ and CD11b suggest that the phospho ERK ½ is present exclusively in the microglia and not in the astrocytes. Western Blot results suggest that there are no alterations in ERK in either MPTP treated animals or controls; however, phospho ERK ½ was found to be significantly increased both in the striatum and SNc in both mouse PD models. Tyrosine hydroxylase (TH) immunolabeling revealed significant decreases in dopaminergic neurons in the SNc in both animal models concomitant with activation of microglia and ERK activation. This study suggests that ERK activation takes place following MPTP treatment and that activation of ERK is occurring primarily in the microglia. The results of this study also suggest that ERK activation may be involved in transcriptional activation of microglia following neurotoxicant insult. Study supported by approved NCTR protocols E751201 and E747701

2336 MPTP Neurotoxicity Is Highly Concordant Between the Sexes in BXD Recombinant Inbred Mouse Strains

G. Alam[4], D. B. Miller[1], J. P. O'Callaghan[1], L. Lu[2,3], R. W. Williams[3] and B. C. Jones[3]. *[1]Centers for Disease Control and Prevention-National Institute for Occupational Safety and Health, Morgantown, WV; [2]Jiangsu Key Laboratory of Neuroregeneration, Nantong University, Nantong, China; [3]University of Tennessee Health Science Center, Memphis, TN and [4]Pharmaceutical Sciences, Northeast Ohio Medical School, Rootstown, OH.*

Continuing our previous work in which we showed wide-ranging strain differences in MPTP neurotoxicity in male mice among ten BXD recombinant inbred strains, we replicated our work in females from nine of the same strains. Mice received a single s.c. injection of 12.5 mg/kg MPTP or saline. Forty-eight hours later the striatum was dissected for neurochemical analysis. Striatal dopamine (DA) and its metabolites, DOPAC and HVA, striatal serotonin (5-HT) and its metabolite, 5-HIAA, were analyzed using HPLC. Tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) were measured using ELISA. There were wide genetic variations in the DA, DOPAC, HVA, TH and GFAP responses to MPTP. We also performed principal component analysis (PCA) on the difference values, saline minus MPTP, for DA, DOPAC, HVA and TH and mapped the dominant principal component to a suggestive QTL on chromosome 1 at the same location that we observed previously for males. Moreover, there were significant correlations between the sexes for the effect of MPTP on DA, HVA, and TH. Our findings suggest that the systems genetic approach as utilized here can help researchers understand the role of sex in individual differences. The same approach can pave the way to understand and pinpoint the genetic bases for individual differences in pathology attributable to toxicants. Such systems genetics approach has broad implications for elucidating gene-environment contributions to neurodegenerative diseases.

2337 Nurr1 Activation Prevents Neurotoxic Injury in the MPTP Model of Parkinson's Disease

S. L. Hammond[2], K. Popichak[2], P. Damale[2], E. Richman[2], L. Hunt[2], S. Safe[3] and R. Tjalkens[2]. *[1]Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO; [2]Center for Environmental Medicine, Colorado State University, Fort Collins, CO and [3]Institute for Biotechnology and Technology, Texas A&M University Health Sciences Center, Houston, TX.*

Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons of the ventral midbrain, associated with inflammatory activation of glial cells. The orphan nuclear receptor Nurr1 (NR4A2) suppresses inflammatory gene expression in glial cells and also positively regulate genes associated with the production/release of dopamine (DA) in neurons. Despite these known functions of Nurr1, an endogenous ligand has yet to be discovered. We previously reported that the phytochemical-based compound, 1,1-bis(3'-indolyl)-1-(p-chlorophenyl) methane (C-DIM12), activates Nurr1 in neural cells, suppresses inflammatory gene expression in primary astrocytes and induces a dopaminergic phenotype in neuronal cultures. In current studies, an *in vivo* approach was undertaken to examine the capacity of C-DIM12 to protect against loss of dopaminergic neurons induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Mice were treated (+/-) MPTP (20 mg/Kg) every four days and (+/-) C-DIM12 (25 mg/Kg) every day for 2-weeks. Motor function was monitored during the course of treatment by open field activity and gait analysis. Preliminary data from this study revealed a significant preservation of TH-positive neurons in the substantia nigra (SN), as determined by design-based (3D) stereological methods, as well as preservation of TH protein levels in the striatum, as determined by immunoblotting. C-DIM12 treatment also inhibited the mRNA expression of multiple neuroinflammatory genes in qPCR array studies. To further examine the cell-specific role of Nurr1 in glial cells and DA neurons in the SN, we used adeno-associated viral (AAV) vectors to selectively overexpress Nurr1 in GFAP (+) astrocytes and DA (+) neurons following intracerebroventricular injections in neonates. Expression of Nurr1 was determined by whole-brain image montaging and CLARITY tissue transmutation. These data suggest that Nurr1 is a direct regulator of dopaminergic function in glial cells as well as neurons and that this receptor may be a viable target for selected small molecule therapeutics. This work was supported by a grant from the Michael J. Fox Foundation (RBT) and by NIH-ES021656 (RBT).

2338 Metabolic Flux Analysis in Human Dopaminergic Neurons Under Toxicant Stress

J. Delp[3], S. Gutbier[3], L. Zhao[2], T. Hartung[2], C. Zasada[1], S. Kempa[1], K. Nöh[4] and M. Leist[3]. *[1]BIMSB Proteomics/Metabolomics, Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany; [2]Center for Alternatives to Animal Testing, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD; [3]Doerenkamp-Zbinden Chair for in vitro Toxicology and Biomedicine, University of Konstanz, Konstanz, Germany and [4]Institut für Bio- und Geowissenschaften, IGB-1: Biotechnologie, Forschungszentrum Jülich GmbH, Jülich, Germany*. Sponsor: M. Leist.

Background: Luhmes cells are non-transformed conditionally immortalized human neuronal precursor cells which can be differentiated to dopaminergic neurons within six days. They are used as a model for (developmental) neurotoxicity. The cells are suitable to be used for both classical viability analysis and the study of neurite outgrowth. Methods: Either proliferating or differentiated (by addition of cAMP, tetracycline and GDNF) cells were used for experiments. Metabolomics analysis was performed using an LC-MS approach. For isotope labeling metabolic flux analysis cells were fed with 13C-labelled glucose or glutamine. Subsequent GC-MS/MS analysis was used to quantify metabolite pools and fluxes. Flux maps resulted from computational modeling based on absolute concentrations and label incorporations into the CCM-metabolites. Results: Upon induced differentiation, Luhmes cells change their phenotype from precursor to fully differentiated dopaminergic neurons, e.g. shown by cell cycle arrest and expression of tyrosine hydroxylase. This change in phenotype is accompanied by a change in concentrations and fluxes of intermediates of the central carbon metabolism (CCM). By the use of stable isotope labelled metabolite precursors, a flux map of the CCM of Luhmes was established for undifferentiated and differentiated cells. The metabolic flux analysis indicated that precursor cells have a stem cell-like metabolism, whereas differentiated cells acquire a neuronal-like metabolism. Based on this set of background data, the metabolic impact of toxic chemicals can be described in high detail for different neuronal differentiation stages.

For instance, substances that specifically inhibited neurite outgrowth, without being cytotoxic, affected central carbon metabolism in a characteristic way. For the neurotoxicant MPP+, the metabolic disturbances were quantified during an early non-symptomatic phase. Compensatory flux regulations like increased utilization of glutamine were observed.

2339 Ziram Inhibits Ubiquitin Activating Enzyme E1 in Human Neurons

M. D. Neely[3], K. E. Dennis[3], M. Moran[3], W. M. Valentine[3] and A. B. Bowman[3]. [1]*Aspirnaut, Vanderbilt Medical Center, Nashville, TN;* [2]*Medicine, Aspirnaut, Vanderbilt Medical Center, Nashville, TN and* [3]*Neurology, Vanderbilt Medical Center, Nashville, TN;* [4]*Pathology, Microbiology and Immunology, Vanderbilt Medical Center, Nashville, TN.*

Parkinson's disease (PD), a chronic, progressive, non-curable neurodegenerative disease is the result of complex interactions between genetic and environmental risk factors. The pathology of PD suggests that the dysfunction of the ubiquitin-signaling-system (USS) may play a role in the etiology of PD. USS is an intricate and extensive cellular regulatory system comprising more than 500 different components that has a role in neuronal viability, proliferation, synaptic plasticity, synapse development, and neuronal transmission. In addition to genetic factors, environmental toxicants affecting the USS have been associated with PD etiology. Residential exposure to dithiocarbamate pesticides has been associated with increased PD risk and a subset of these chemicals cause dopamine (DA)-neuron specific toxicity and inhibit E1 activating enzyme (E1), the first of a cascade of enzymes composing the USS. Here we assess and compare the effects of Ziram, a dithiocarbamate pesticide, on cortical glutamatergic and midbrain dopaminergic neurons differentiated from human-induced pluripotent stem cells. Somewhat surprisingly, human cortical glutamatergic neurons appeared more sensitive than their mesencephalic dopaminergic counterparts in assessments using a resazurin-reduction based cell survival assay. Ziram at non-neurotoxic exposures reduces the ubiquitination of ubiquitin-activating enzyme E1 in both these neuronal lineages with a similar dose response curve as demonstrated by Western blotting. In addition, preliminary observations suggest a dose and time-dependent effect of Ziram on tyrosine hydroxylase protein expression levels in the dopaminergic neurons. Our observations suggest that Ziram potently affects E1 enzyme, and thus the USS in human neurons. We are evaluating the effects of Ziram on targets downstream of E1 to gain a better understanding of Ziram-induced neurotoxicity in human neurons of different lineages. Supported by P30 ES000267 Pilot Project (MDN), ES010563 (ABB) and ES019969 (WMV).

2340 *In Vitro* Toxicity and Resilience of A 3D Human Dopaminergic Model to Rotenone

G. Harris, J. Delp, D. Pamies, H. Hogberg, T. Hartung and L. Smirnova. *CAAT, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Anastasis, greek for resurrection, has recently come into play in understanding cellular mechanisms of recovery and reversal of apoptosis. This concept is not implemented in current *in vitro* toxicological testing, however is relevant to mechanistic toxicology. We propose that cellular response to environmental stress differs not in how cells are hit but how they recover. We hypothesize that not the primary hit, but the ability of the cells to cope with the stress (cellular resilience) determines organ selectivity. Toxic exposures can lead to acute adverse effects, or, in a long-term perspective, either to cellular resilience or delayed adverse effects/disease. Here, we expose an *in vitro* 3D dopaminergic model to known mitochondrial toxicant rotenone, to compare molecular perturbations after primary hit (day 8) and delayed effects 7 days after compound wash-out (day 15). In contrast to currently performed animal studies (high dose), we aim to study long-term effects after low-dose exposures. To assess differences in acute and delayed cellular responses, we treated aggregates with 0.1 uM rotenone and assessed changes in expression for energy metabolism and stress response genes (ATF4, ASS1, CBS, CTH, MLF1IP, SHMT and TYMS) and mitochondria/apoptosis-related miRNAs (miRs 7, 16 and 210). We observed a decrease in TYMS and MLF1IP expression after exposure to rotenone, which was further decreased on day 15, while ASS1, CTH. SHMT expression which was upregulated on day 8, returned to control levels on day 15. In the same experiments, miR-16 (apoptosis) and miR-210 (mitochondrial function) were unchanged on day 8 and day 15. However, miR-7, known to play a role in Parkinson's disease, was significantly downregulated after acute exposure and upregulated on day 15. With these results, we aim to further study the role of miR-7 in neurotoxicity/neuroprotection and long-term dopaminergic neurodegeneration, and cell survival pathways contributing to recovery and/or resilience.

2341 Mitochondrial Neurotoxic Pesticides Promote Epigenetic Dysregulation by Histone H3 and H4 Hyperacetylation in Dopaminergic Neuronal Model of Parkinson's Disease

A. Charli, H. Jin, V. Anantharam, A. Kanthasamy and A. G. Kanthasamy. *Interdepartmental Graduate Program in Toxicology and Department of Biomedical Science, Iowa State University, AMES, IA.*

Growing evidence implicates continual exposure to environmental pesticides in the etiopathogenesis of Parkinson's disease (PD). Environmental neurotoxic pesticides can induce mitochondrial dysfunction and increase oxidative stress, which are key pathological hallmarks of PD. Previously, we demonstrated that dysregulation of histone acetylation homeostasis can increase susceptibility of dopaminergic neurons to degenerative processes. Using confocal microscopy and seahorse bioanalyzer, we showed that exposing an N27 dopaminergic neuronal cell model of PD to the greenhouse pesticides tebufenpyrad and pyridaben induced significant neurotoxicity by affecting mitochondrial structure and function. In this study, we examined whether tebufenpyrad- and pyridaben-induced mitochondrial dysfunction influences epigenetic histone acetylation. Interestingly, N27 dopaminergic cells exposed to 3 μM of tebufenpyrad or pyridaben showed a time-dependent increase in the levels of histone H3 and H4 acetylation. The classic mitochondrial complex-1 inhibitor rotenone also induced histone H3 and H4 acetylation. Immunocytochemical analysis revealed that tebufenpyrad, pyridaben and rotenone exposure preferentially acetylate lysine sites K24 and K5 on H3 and H4, respectively. 3D-reconstruction of confocal microscopy images performed using IMARIS displayed time-dependent architectural changes in mitochondria and nuclear morphology. Notably, treating dopaminergic neuronal cells with the histone acetyl transferase (HAT) inhibitor anacardic acid (8.5 μM) significantly protected against tebufenpyrad- and pyridaben-induced H3 and H4 acetylation as well as neurotoxicity. Collectively, our data reveal a novel interplay between neurotoxic pesticide-induced mitochondrial defects and epigenetic acetylation of core nuclear histones, which together may play a critical role in the etiology of environmentally-linked Parkinson's disease. (Supported by NIH grants ES10586 and NS074443).

2342 Investigating the Synergistic Effects of Chlorpyrifos and Cadmium Neurotoxicity in Alpha-Synuclein Overexpressing Dopaminergic Cell Model of Parkinson's Disease

M. Huerta and G. Kwakye. *Neuroscience, Oberlin College, Oberlin, OH.*

Parkinson's disease (PD) is a progressive neurological disorder characterized by selective loss of dopaminergic cells in the substantia nigra of the brain that results in motor, cognitive, and psychiatric abnormalities. Alpha-synuclein (α-syn) is a protein predominantly expressed in the brain and comprises about 60% of cytoplasmic inclusions called Lewy bodies, which are often present in the substantia nigra of PD patients. However, the native function remains unclear. Previous studies have implicated chlorpyrifos (CPF) and cadmium (Cd), two neurotoxins, in causing oxidative stress, protein aggregation and mitochondrial dysfunction in PD. Recognizing some of the shared similarities in the cellular mechanisms between PD, pesticides, and heavy metal neurotoxicity, we hypothesized that CPF, Cd, and native α-syn will exhibit a gene-environment interaction, wherein CPF and Cd co-exposure will synergistically interact and enhance neuronal loss in α-syn expressing cells compared to vector (VEC) controls. Furthermore, we examined the synergistic effect of CPF and Cd co-exposure on native human α-syn. Utilizing an established immortalized rat mesencephalic cell line that overexpresses human native α-syn or empty vector control, we examined the effect of CPF, Cd, and CPF-Cd neurotoxicity using a mitochondrial-dependent cell viability assay (MTT 3-4,5-Dimethylthiazol-2-yl-2,5-Diphenyltetrazolium Bromide Assay). We report that expression of human native α-syn enhances susceptibility to Cd and CPF-Cd interaction following 24 hours exposure. Furthermore, we investigated the contribution of oxidative stress pathways in the synergistic effect between α-syn and CPF-Cd with an oxyblot technique that detects carbonyl group formation on protein chains as well as real-time free radical production detection assay. We report differential rates of protein oxidation and production of free radicals following Cd, CPF, and CPF-Cd exposures. In summary, we have identified a selective response of human native α-syn to CPF, Cd, and CPF-Cd combination. Our data suggests that neurotypical individuals that express native α-syn and potentially exposed to Cd or CPF-Cd may experience neuronal dysfunction and loss regardless of the genetic mutation. This study further establishes the need for future research to elucidate potential adverse effects of multiple environmental pollutant exposures in neurodegenerative diseases.

2343 Blocking Mitochondrial Fission Is Protective Against Paraquat-Induced Neurotoxicity

R. B. Nisr and K. Tieu. *Peninsula Schools of Medicine and Dentistry, Plymouth University, Plymouth, United Kingdom.*

Advances in the genetics of Parkinson's disease (PD) have now established a strong genetic link between mitochondrial dysfunction and PD. Furthermore, these genetic studies have highlighted mitochondrial dynamics (fission / fusion / movement) as a potential therapeutic target for PD. However, PD-linked mutations still represent less than 10% of all PD cases. It is not clear whether perturbed mitochondrial dynamics is also involved in sporadic PD. Although the causes of sporadic PD remain unknown, neurotoxic molecules found in the environment have been implicated. Epidemiological studies suggest the herbicide paraquat (PQ) as a potential agent for increasing risk of developing PD. PQ is a redox cycler that generates superoxide which also impair mitochondrial function. This study test the hypothesis that excessive mitochondrial fission and neurotoxicity induced by oxidative stress generated by PQ could be attenuated by blocking mitochondrial fission. To this end, we treated differentiated dopaminergic neuronal cells (LUHMES) with varying doses of PQ in the presence or absence of treatments that blocked the function of the mitochondrial fission protein dynamin-related protein (Drp1). Our results show that PQ induced severe mitochondrial fragmentation. To quantify real-time mitochondrial respiration in live cells, we used the XFe96 Extracellular Flux Analyser. In a dose- and time-dependent manner, PQ reduced mitochondrial respiratory capacity, reduced coupling efficiency and increased proton leak. This mitochondrial impairment was consistent with the observations of collapsed mitochondrial membrane potential and increased cell death. More importantly, blocking mitochondrial fission via Drp1 inhibition significantly improved mitochondrial function and cell viability against PQ neurotoxicity.

2344 The Legacy Pesticide Dieldrin and Paraquat Decrease Maximum Respiratory Capacity in Rat Dopaminergic N27 Cells

J. T. Schmidt and C. J. Martyniuk. *Physiological Sciences, University of Florida, Gainesville, FL.* Sponsor: N. Denslow.

It is now widely recognized that the mitochondria is a direct target of chemicals in our environment. The vulnerability of mitochondria is dependent upon factors that include epigenetics, tissue-specific regulation, and the age of the animal. Toxicants can impact mitochondrial function through various mechanisms (e.g., mtDNA damage, electron transport chain inhibition/uncoupling, reactive oxygen species generation, among others). Thus, high throughput *in vitro* bioassays are required to assess mitochondrial function under these different conditions. Dieldrin (DLD) is a legacy organochlorine pesticide that bioaccumulates in tissues, is detectable in human blood, and has established epidemiological links to Parkinson's disease. Paraquat (PQ2+) is a model pesticide that targets dopaminergic cells and induces apoptosis. To better determine how DLD and PQ2+ affect mitochondrial function in dopaminergic neurons, we measured oxidative phosphorylation in a mitochondrial stress bioassay to test the hypothesis that both DLD and PQ decrease *in vitro* oxygen consumption rate of mitochondria in the rat immortalized dopaminergic N27 cell line. N27 cells were treated for 24 hours at one of the following doses: 2.5 and 25 µM DLD or PQ2+. N27 cells were also incubated at 250 µM PQ2+. Cells were seeded overnight and incubated with pesticides the following day. Cells were washed and analyzed using a Seahorse XFe24 extracellular flux analyzer in assay media. Repeated measures analysis on respiratory rates revealed that N27 cells treated with 25 µM DLD showed lower maximum respiration compared to control N27 cells following treatment with the uncoupling agent carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) ($P<0.05$). The pesticide PQ2+ suppressed maximum respiration of N27 cells following FCCP treatment at 25 and 250 µM ($P<0.05$). Both pesticides at doses >25 µM reduced "spare respiratory capacity" of the mitochondria, the difference between ATP produced by oxidative phosphorylation at basal and that at maximal activity. Proton leak was not affected by these pesticides in these experiments. Thus, N27 rat dopaminergic cells exposed to DLD and PQ2+ demonstrate impaired mitochondrial function, most notable effects on the maximum respiratory capacity of these cells following stress. Future efforts are address the mechanisms underlying this pesticide-induced mitochondrial dysfunction for different neuroactive pesticides.

2345 Neonatal Systemic Exposure to Lipopolysaccharide Enhances Adult Vulnerability to Paraquat Neurotoxicity

L.-T. Tien[2], Y. Pang[1], S. Lu[1], J. W. Lee[1], S. Lin[1], A. J. Bhatt[1], R. D. Savich[1] and L.-W. Fan[1]. [1]*Department of Pediatrics, Division of Newborn Medicine, University of Mississippi Medical Center, Jackson, MS and* [2]*School of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan.*

We have previously shown that neonatal intracerebral injection of lipopolysaccharide (LPS) increases the risk of rotenone (a commonly used pesticide)-induced dopaminergic damage in adult rats. This study was designed to further test whether neonatal systemic LPS exposure also increases the vulnerability of adult dopaminergic neuron to paraquat, a widely used herbicide. LPS (2 mg/kg) was administered intraperitoneally into postnatal day 5 (P5) rats. On P70, rats were challenged with paraquat through subcutaneous mini-pump infusion at a dose of 0.3 mg/kg per day for 14 days. This paraquat treatment regimen ordinarily does not produce toxic effects on behaviors in normal adult rats. However, LPS pre-exposed rats developed Parkinson's disease-like motor neurobehavioral impairments after paraguat treatment, including bradykinesia (prolongation of the movement time), akinesia (prolongation of the reaction time), and rigidity (increase in muscle tone or magnitude of stretch reflexes). Structural examination of the nigrostriatal pathway revealed that neonatal LPS exposure enhanced paraquat neurotoxicity to cause a significant loss of tyrosine hydroxylase immunoreactive neurons in the substantia nigra, and decrease in retrogradely labeled nigrostriatal dopaminergic projecting neurons of rats. Our results indicate that perinatal brain inflammation may cause the nigrostriatal system in the adult brain more vulnerable to being damaged by environmental toxins at an ordinarily non-toxic or sub-toxic dose to develop Parkinson's disease-like motor dysfunction and pathological features.

2346 Neurochemical Perturbations and Dopaminergic Injury Following Short-Term Inhalation Exposure to the Oil Dispersant COREXIT® EC9500A

K. Sriram, G. X. Lin, A. M. Jefferson, W. T. Goldsmith, M. Jackson, W. McKinney, J. S. Fedan and D. G. Frazer. *CDC-NIOSH, Morgantown, WV.*

Consequent to the 2010 Deepwater Horizon oil spill, there are concerns about the short- and long-term adverse health effects of exposure to crude oil, weathered-oil products and oil dispersants. Approximately, 1.84 million gallons of dispersants were applied to contain the spill, of which 58% was applied on the surface. COREXIT® EC9500A (CE) was the predominant dispersant used to enhance the oil biodegradation rate. As surface treatment with dispersants involved aerial application there is a likelihood that the response workers may have been at risk for exposure, primarily via inhalation. Health Hazard Evaluation surveys conducted by NIOSH suggested that the workers may have experienced abnormal psychosocial symptoms like depressed feeling and mood alterations. Unfortunately, as a significant number of workers who experienced health symptoms were likely exposed to both crude oil and dispersant, the health effects of dispersant alone were difficult to discern. To determine if CE per se poses a neurological risk, rats were exposed by whole-body inhalation to CE (27 mg/m3) either acutely (5 h/d x 1 d) or for a short-term (5 h/d x 9 d). After 1 or 7 days, changes in synaptic proteins, biogenic neurotransmitters and their receptors were evaluated because synaptic disruption and imbalances in dopamine (DA) and serotonin (5-HT) are known to be associated with depression, lack of coordination and short-term memory. Acute exposure to CE altered several axonal and synaptic proteins in discrete brain areas, indicative of aberrant neurotransmitter signaling. Short-term exposure elicited small but significant decrements (15-30 %; $p<0.05$) in DA, 5-HT and norepinephrine in the striatum, frontal cortex and hypothalamus. CE also altered Drd1, Drd2, and Htr1a receptor mRNAs in the striatum and frontal cortex. In addition, CE reduced striatal tyrosine hydroxylase (≈ 20 %; $p< 0.05$), while increasing Gfap mRNA (1.6-fold; $p<0.05$) and protein (23 %; $p<0.05$), suggestive of dopaminergic injury. Whether such changes will persist and cause progressive dopaminergic neurodegeneration remains to be elucidated.

2347 The "Bath Salts" Constituent 3,4-Methylenedioxypyrovalerone (MDPV) Induces Cytotoxic Effects on Human Dopaminergic SH-SY5Y Cells

S. Ali[4], H. Rosas-Hernandez[3,4], E. Cuevas[4], S. M. Lantz-McPeak[4], B. L. Robinson[4], S. Z. Imam[4], M. G. Paule[4], C. Gonzalez[3], K. C. Rice[2], B. M. Gannon[1] and W. E. Fantegrossi[1]. *[1]Dept. Pharmacol. & Toxicol., UAMS, Little Rock, AR; [2]Drug Design & synthesis Section, Chemical Biology Research, NIDA/NIAAA, Bethesda, MD; [3]Facultad de Ciencias Quimicas, Laboratorio de Fsiologia Celular, UASLP, San Luis Potosi, SLP, Mexico and [4]Neurochemistry Laboratory, Division of Neurotoxicology, NCTR, Jefferson, AR.*

Synthetic cathinones are a rapidly growing group of psychostimulant drugs usually referred to as "bath salts" (BS). Although many different substances are present in BS, 3,4-methylenedioxypyrovalerone (MDPV) is perhaps the most prevalent psychoactive constituent. Many psychostimulant drugs exert neurotoxic effects by altering central monoamine systems, typically increasing extracellular levels of dopamine, serotonin and norepinephrine via interactions with various reuptake transporters. In addition, it has been reported that MDPV induces apoptosis in the neonatal mouse brain by increasing the expression of caspase-3. The reports to date suggest that MDPV may induce neurotoxic effects and alter brain dopaminergic systems; however, no studies have previously assessed the potential toxicity of this drug in human dopaminergic cells. The aim of this study was therefore to evaluate the effects of MDPV on the human dopaminergic cell line SH-SY5Y. Cells were maintained in MEM/F12 medium and treated with MDPV (1-250 μM) for 24 hours. LDH release was measured as an index of cytotoxicity, DCFH-DA oxidation was assessed as an index of ROS production, bromodeoxyuridine (BrdU) incorporation was quantified as an index of cellular proliferation and DNA fragmentation was gauged as an index of apoptosis. The results indicate that MDPV increased production of ROS at 1 and 3 hours post-treatment at all concentration used, however, ROS production was not different from control at 6 and 24 hours post-treatment. MDPV also induced a decrease in cellular proliferation at concentrations of 100 and 250 μM, which correlates well with DNA fragmentation results as complementary markers of cell death. In contrast, MDPV had no effects on LDH release at any tested concentration. These results suggest that MDPV induces dopaminergic neurotoxicity, however, further studies will be required to determine the mechanisms involved in this process.

2348 M1 to M2 Microglial Switch Accompanies Cessation of Progressive Dopaminergic Cell Loss Following Lipopolysaccharide Exposure

E. Beier[2] and J. Richardson[1,2]. *[1]Northeast Ohio Medical University, Rootstown, OH and [2]Rutgers University, Piscataway, NJ.*

Systemic inflammation has been suggested to induce neurodegeneration through activation of inflammatory cells within sensitive brain regions in a number of neurodegenerative diseases, including Parkinson's disease (PD). However, less is known about the role of microglial phenotypes (i.e. M1 and M2) that may contribute to, or modulate, cell loss during progression of the disease. Repeated exposure to LPS (1 mg/kg for 4 days) has been shown to cause a progressive loss of dopamine neurons by two weeks after cessation of exposure, but not at 1 day after exposure. Here, we sought to determine whether LPS-induced dopaminergic cell loss continued past this time point and assess the role of microglial phenotypes in the process. LPS administration caused a 32.3% loss of dopamine neurons two weeks after exposure, but did not result in significantly higher cell loss at 4 weeks following LPS (38% cell loss). Initially, determination of microglial marker expression in the striatum at 1 day after cessation of LPS by qPCR suggested a strong M1-like response. This was characterized by increased tumor necrosis factor-α (4.8 fold), inducible nitric oxide synthase (2.0 fold), interleukin-1 beta (IL-1β, 8.9 fold), IL-6 (10.7 fold), and monocyte chemotactic protein 1 (4.5 fold) expression. Two weeks following cessation of exposure, there was a reduction of M1-like markers and a rise in M2-like gene expression, Ym1 (1.8 fold) and arginase (2.6 fold). After four weeks, there was also an increase in mannose receptor, c type 1 (2.5 fold) and IL-10 (2.1 fold). Our findings suggest that M1-like microglial activation by LPS produces a cytotoxic effect that leads to progressive SN neurodegeneration. Furthermore, these data suggest that an M1-M2 shift in activity may reduce the progressive inflammation and arrest dopaminergic cell loss. Supported in part by NIH T32ES007048, P30ES005022, R01ES021800, and the Michael J Fox Foundation.

2349 Longterm Neuroprotection of Decellularized Brain Extracellular Matrix Containing bFGF by Intracerebral Transplantation in a Rat Parkinson's Disease Model

Q. Lin. *University of Louisville, Louisville, KY*. Sponsor: Y. Tan.

Basic fibroblast growth factor (bFGF) is widely studied as a potential agent for central nervous system (CNS) diseases, especially for Parkinson's disease (PD). However, the poor stability and short half-life of bFGF hampers its clinical use. As a biologic scaffold, extracellular matrix (ECM) is recommended for tissue regeneration in preclinical and clinical settings. In this study, CNS-derived biologic scaffold containing bFGF was used to enhance and extend the neuroprotective effect of bFGF and improve the efficiency of PD targeted therapy. Decellularized brain ECM (dcBECM) was prepared by chemical extraction. The biocompatibility of dcBECM was evaluated using CCK-8 assay and magnetic resonance imaging (MRI). The controlled-release behavior of dcBECM containing bFGF (bFGF+dcBECM) was confirmed by ELISA. Furthermore, the cytocompatibility and neuroprotective effect of bFGF+dcBECM were evaluated *in vitro* and *in vivo*. The results demonstrated that dcBECM preserved a three-dimensional network structure with high biocompatibility. MRI of dcBECM implanted rats showed nearly seamless fusion of dcBECM with the adjoining tissues. The cumulative release rate of bFGF+dcBECM *in vitro* reached to 75.88% at 10.0 h and maintained sustained release during the observation. ELISA results *in vivo* further confirmed the controlled-release behavior (from 12h to 3d) of bFGF+dcBECM in brain tissues. Furthermore, bFGF+dcBECM treatment enhanced the cell survival in a PD cell model and promoted the expression of nerve regenerative proteins in PD rats. In conclusion, sustained neuroprotection in PD rats was achieved using bFGF+dcBECM. This therapeutic strategy is promising to be an effective, efficient and safe alternative for CNS disease treatment. Keywords: Decellularization; Brain extracellular matrix; Basic fibroblast growth factor; Parkinson's disease; Neuroprotection.

2350 Environmental Neurotoxicant Manganese Alters Exosomal miRNAs and Autophagic Regulation in Cell Culture Model of Parkinson's Disease

D. Harischandra[1], M. Zamanian[2], D. Rhokad[1], H. Jin[1], V. Anantharam[1], M. Kimber[1], A. Kanthasamy[1] and A. Kanthasamy[1]. *[1]Iowa State University, Ames, IA and [2]Northwestern University, Evanston, IL.*

Many age-related neurodegenerative disorders share a common pathogenic mechanism involving the aggregation and deposition of misfolded proteins. In Parkinson's disease (PD), the accumulation of misfolded and aggregated α-synuclein (αSyn) is considered a key pathophysiological feature. These misfolded proteins can be degraded by autophagy via the lysosomal pathway. However, the lysosomal degradation pathway is impaired in the disease state, leading to a significant accumulation of autophagic vesicles in the neuronal body. Given the role of the divalent metal manganese (Mn) in PD-like neurological disorders, we characterized the effect of Mn on misfolding and its role in autophagic/lysosomal dysfunction using an MN9D dopaminergic cell model of PD, which stably expresses wild-type human αSyn. Western blot analysis revealed that Mn increased the expression of the autophagosomal markers LC3-II and Beclin-1, whereas the lysosomal marker LAMP2 was downregulated in αSyn-expressing cells relative to vector control cells, suggesting that Mn treatment impairs the autophagic/lysosomal degradation pathway. Interestingly, Mn treatment also induced the release of αSyn-containing exosomes into the extracellular media, as determined by NanoSight particle analysis and electron microscopy. Furthermore, we found these αSyn-containing exosomes are bioactive and able to induce neuroinflammatory response and neurodegeneration in cell culture models of PD. To further elucidate the molecular mechanisms underlying Mn-induced autophagic/lysosomal dysregulation, we performed next-generation miRNA sequence analysis of manganese- and vehicle-stimulated exosomes. We identified 43 miRNAs differentially expressed in Mn-stimulated αSyn exosomes as compared to control exosomes. Among them, 12 mRNAs were associated with regulation of autophagic/lysosomal degradation pathway. Three miRNAs (miRNAs-124, 320a and 325) were previously reported to control autophagic regulation by targeting Bim, Hsc70 and E2F1 in experimental models of PD. Collectively, our results suggest a novel paradigm in which dysregulation of exosomal miRNA pertaining to autophagic degradative machinery may play a role in cell-to-cell transmission of misfolded αSyn protein. (NIH ES19267, ES10586, and NS088206)

2351 Moleclar Mechanisms of Manganese-Induced Exosome Release and Its Relevance to Synucleopathies

D. Rokad, D. S. Harischandra, H. Jin, V. Anantharam, A. Kanthasamy and A. G. Kanthasamy. *Interdepartmental Toxicology Graduate Program and Department of Biomedical Science, Iowa State University, Ames, IA.*

Metal exposure increases risk of chronic neurological diseases including Parkinson's, mainly characterized by progressive accumulation of aggregated α-synuclein protein. Oligomeric α-synuclein can be released from neurons via exosomes, enabling misfolded α-synuclein to propagate to neighboring cells. Our recent studies revealed that manganese greatly increases the release of exosomes containing aggregated α-synuclein in dopaminergic neurons. The key cellular-molecular signaling mechanisms driving manganese-induced exosomes release remain unknown. Herein, we examined the role of manganese in modulating endosomal protein trafficking to promote α-synuclein exosomal release. MN9D dopaminergic neuronal cells stably expressing human wild-type α-synuclein were treated with or without 300-μM manganese for 24h and the expression of Rab11a, a key Rab GTPase regulating endosomal recycling, was examined by Western blot. Manganese significantly reduced Rab11a expression, suggesting that the metal disrupts endosomal-recycling mechanisms, thereby forcing endosomes to mature into multivesicular bodies (MVBs). Interestingly, manganese exposure upregulated Rab27a, the key Rab GTPase that promotes exosomes release through fusion of MVBs with the plasma membrane. To further investigate whether manganese facilitated α-synuclein exosomal release by impairing the retromer sorting pathway that targets MVBs for trans- Golgi recycling, we used both qRT-PCR and immunocytochemistry to show that manganese exposure downregulated the mRNA and protein expression of Vps35, the key component of retromer complex, indicating manganese impairs retrograde endosomal transport to Golgi for recycling. Taken together, these novel findings demonstrate that manganese impairs endosomal trafficking and leads to retromer dysfunction, thereby promoting MVB formation and exosomal release of misfolded α-synuclein in a dopaminergic cell model of Parkinson's Disease. (NIH grants ES19267, ES10586, NS74443 and Linda Lloyd Endowed Chair)

2352 Astroglial Mortalin Is Decreased in the Striatum of Manganese-Exposed Mine Workers and Enhances Neurotoxicity

T. J. Cook[1,5], J. G. Hoekstra[5], T. Stewart[5], K. K. Canales[5], P. Ho[5], A. A. Salvador[5], L. F. Gonzalez-Cuyar[5], G. Nelson[6], B. A. Racette[4,6], H. Checkoway[3], D. L. Eaton[5] and J. Zhang[5]. *[1]Gradient Corporation, Seattle, WA; [2]Department of Environmental & Occupational Health Sciences, University of Washington, Seattle, WA; [3]Department of Family Medicine and Public Health, University of California San Diego, La Jolla, CA; [4]Department of Neurology, Washington University, St. Louis, MO; [5]Department of Pathology, University of Washington, Seattle, WA and [6]School of Public Health, University of Witwatersrand, Parktown, South Africa.*

Despite similar clinical phenotypes, unlike idiopathic Parkinson's disease (iPD), manganese (Mn) over-exposure primarily leads to basal ganglial, rather than nigral, neurotoxicity. Here, we studied basal ganglia levels of astroglial mortalin, an essential mitochondrial chaperone protein that is decreased in the nigra of iPD patients. Using human brain tissue specimens from deceased mine workers, we identified lower levels of astroglial mortalin in the corpus striatum, but not the nigra, of Mn-exposed workers utilizing quantitative fluorescence microscopy. We subsequently used an *in vitro* primary astrocyte-neuron co-culture system to further investigate the consequence of reduced astroglial mortalin on neuronal health in the context of Mn exposure. It was found that striatal neurons cultured in the presence of astrocytes expressing reduced levels of mortalin were more sensitive to Mn treatment (10 mM) than those expressing endogenous levels, observed as a decrease in the number of presynaptic terminals. Functional assessments of astrocytes revealed endoplasmic reticulum and oxidative stress, impaired intracellular calcium signaling, and an increased presence of aggregated proteins in Mn-treated astrocytes with mortalin knocked down, and these effects were mitigated in astrocytes lacking expression of α-synuclein. We propose that these effects, taken together, suggest a critical role for astroglial mortalin in Mn-induced neurotoxicity, and involves mechanisms related to astrocytic cellular processes intimately associated with endogenous α-synuclein.

2353 Loss of Striatal Cholinergic Interneurons in the Presence of Intact Nigral Dopaminergic Neurons in Early Manganese-Induced Parkinsonism

K. K. Gonzales[2], J. McGlothan[2], K. Stansfield[2], J. Schneider[1] and T. Guilarte[2]. *[1]Thomas Jefferson University, Philadelphia, PA and [2]Mailman School of Public Health, Columbia University, New York, NY.*

Movement abnormalities caused by chronic manganese (Mn) intoxication clinically resemble but are not identical to those in idiopathic Parkinson's disease (iPD), a disorder linked to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Clinical evidence from individuals with Mn-induced parkinsonism has revealed normal dopaminergic marker levels in the striatum, along with an unresponsiveness to the anti-parkinsonian dopaminergic drug levodopa (Perl & Olanow, 2007; Huang et al., 2015). In agreement, pre-clinical data from our laboratory have shown markedly impaired dopamine release from intact nigrostriatal dopaminergic axon terminals in Mn-exposed monkeys using Positron Emission Tomography (PET) (Guilarte, 2006, 2008). However, in-depth pathological studies as performed in iPD patients and parkinsonian animal models are still needed for Mn-induced parkinsonism. Through stereological analyses, we recently reported for the first time that the total number of tyrosine hydroxylase (TH)-positive neurons (N; mean ± S.E.M.) in the SNpc did not differ significantly between control (4 animals; N=240,569 ± 14,943) and Mn-exposed groups (5 animals; N=280,532 ± 13,840) (t-test; $p = 0.07$). Because of the strong functional relationship between striatal cholinergic interneurons and nigrostriatal dopaminergic afferents, we next investigated the effects of Mn intoxication on the total number of neurons immunolabeled with choline acetyltransferase (ChAT) in the striatum. Preliminary findings demonstrate a marked loss of cholinergic interneurons throughout the entire rostrocaudal extent of the putamen of three Mn-exposed monkeys in comparison to one control animal. These collective findings suggest that the motor deficits and impaired striatal dopamine release in early Mn-induced parkinsonism are unrelated to dopaminergic nigral cell loss but may be influenced by substantial degeneration of cholinergic interneurons in the putamen. Current studies are investigating the absolute number of striatal ChAT-positive neurons in nine additional monkeys.

2354 Reversibility of Effects of Manganese Toxicity: A Longitudinal Neuroimaging Study on Welders

U. Dydak[2,3], R. Ma[3], C.-L. Yeh[3], E. Cameron[3], D. A. Edmondson[3], S. E. Zauber[2], S. Snyder[3] and E. Ward[3]. *[1]Department of Neurology, Indiana University School of Medicine, Indianapolis, IN; [2]Dept. of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN and [3]School of Health Sciences, Purdue University, West Lafayette, IN.*

Occupational exposure to manganese (Mn) in welding fumes can lead to subtle effects on cognitive and motor function, most likely mediated by changes in neurotransmitter concentrations. In particular, we have shown previously that increases of gamma-aminobutyric acid (GABA) in the thalamus were not only associated with motor function scores, but also with Mn exposure over the past 3 months as well as Mn concentration in the substantia nigra (SN), as measured by the R1 relaxation rate in MRI. Yet, little is known about the reversibility of these GABA changes with changing exposure. Following the Mn exposure of our welder cohort with personal air sampling over two years, 14 welders and 4 controls have been tested a second time using MRI and GABA-edited magnetic resonance spectroscopy. Mean past-3-months Mn exposure (prior to MRI measurements) of the welders had significantly decreased from 0.029 to 0.017 mg/m3-yr ($p< 0.01$). As expected, R1 relaxation rate, indicative of brain Mn accumulation, decreased in all regions with highest decreases in SN (-27.5%, $p<0.0001$, paired t-test), and a significant association between the R1 changes and changes in past-3-months exposure in the left frontal cortex ($R=0.75$, $p<0.01$). Yet, a significant difference in R1 between welders and controls still remains in most brain regions ($p<0.01$, whole-brain analysis). In agreement with these changes, thalamic GABA levels of welders have significantly decreased ($p<0.01$, paired t-test) back to control levels. Changes in GABA levels are significantly associated with changes in past-3-months ($R=0.69$, $p<0.01$). Finally, no grey matter volume changes at $p<0.001$ were found in welders at either time point, consistent with no cell loss occurring at this stage. In summary, our results strongly support that thalamic GABA levels are reversible with decreasing exposure and decreasing brain manganese levels. (Supported by NIEHS R01 ES020529)

2355 Parkinson's Disease versus Manganese Toxicity: A Neuroimaging Comparison

D. A. Edmondson[3], C.-L. Yeh[3], E. Azizi[3], R. Ma[3], E. Ward[3], S. Snyder[3], S. E. Zauber[2] and U. Dydak[2,3]. [1]*Department of Neurology, Indiana University School of Medicine, Indianapolis, IN;* [2]*Dept. of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN and* [3]*School of Health Sciences, Purdue University, West Lafayette, IN.* Sponsor: U. Dydak.

It is well established that manganese (Mn) toxicity can cause a form of parkinsonism. Using MRI and magnetic resonance spectroscopy, we compared the following neuroimaging biomarkers in Mn-exposed welders and idiopathic Parkinson's Disease (IPD) patients: R1 relaxation time (indicative of brain Mn concentrations), R2* relaxation time (indicative of brain iron (Fe) content, but also affected by Mn) and concentration changes of the neurotransmitter γ-aminobutyric acid (GABA) in the thalamus. 32 welders (15 high-exposed, mean airborne Mn = 0.23 mg/m3; 17 low-exposed, mean airborne Mn =0.09 mg/m3) were compared to 23 matched controls working at the same factory; and 19 IPD patients were compared to 18 matched controls. Only the welder group showed significantly increased R1 values across many brain regions (all $p<0.001$), particularly in the globus pallidus (GP), indicative of elevated Mn levels. Significantly increased R2* levels were found in the SN in IPD patients, in agreement with literature, and in the GP in both IPD patients and welders ($p<0.001$). Correcting for age, we find that R2* in the GP is significantly higher in welders compared to IPD patients ($p<0.05$). To control for the effect of Mn on R2* a partial correlation analysis controlled for R1 was performed. In welders, elevated R2* in the GP is strongly associated with years of welding ($p<0.001$), individual Mn air levels ($p<0.01$) and individual Fe air levels ($p<0.01$). In IPD, an association of increased R2* with UPDRS motor scores, indicative of disease severity, was found in the SN ($p<0.05$) but not in welders. Finally, elevated thalamic GABA levels, found in high-exposed welders ($p<0.01$), were also reproduced in IPD patients ($p=0.016$), with the thalamic GABA levels in IPD not being significantly different from high-exposed welders. In both welders and IPD patients, elevated thalamic GABA is associated with increased inhibition as measured by reaction times in the Simon task ($p<0.05$). Using this comparison to IPD, we conclude that changes in R1 and R2* in welders are markers of Mn and Fe exposure, with R2* governed by both Mn and Fe, while elevated thalamic GABA levels are a marker of effect, most likely of motor impairment. (Supported by NIEHS R01 ES020529)

2356 The Effect of Manganese Exposure on ATP13A2-Deficient Mice

N. Santiago[4], E. Mullin[6], S. Pampohile[4], O. Ekhator[4], S. Linn[1], J. Holden[4], D. Lemkuhl[4], S. Karkare[4], D. Aga[6], J. Roth[6], B. Liou[5], Y. Sun[5], G. Shull[4], P. Schultheis[1] and S. Fleming[4]. [1]*Biology, Northern Kentucky University, Highland Heights, KY;* [2]*Chemistry, University of Buffalo, Buffalo, NY;* [3]*Molecular Genetics, University of Cincinnati, Cincinnati, OH;* [4]*Neurology, University of Cincinnati, Cincinnati, OH;* [5]*Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH and* [6]*Pharmacology and Toxicology, University of Buffalo, Buffalo, NY.* Sponsor: J. Richardson.

Loss of function mutations in the P5-ATPase ATP31A2 are associated with Kufor-Rakeb Syndrome and Neuronal Ceroid Lipofuscinosis. ATP13A2's function is unclear, but *in vitro* studies suggest it is a lysosomal protein that interacts with the metals manganese (Mn) and zinc (Zn) and the presynaptic protein alpha-synuclein (aSyn). *In vivo*, loss of ATP13A2 function in mice causes sensorimotor deficits, enhanced autofluorescent storage material, and in one study accumulation of aSyn. In the present study, wildtype (WT) and ATP13A2-deficient (13a2) mice were treated with saline or Mn at 5-9 or 12-19 months of age. Sensorimotor function was assessed beginning on day 30. In the brain, autofluorescence was measured in the cortex, hippocampus, substantia nigra, and cerebellum. Soluble and insoluble aSyn protein levels were determined in the dorsal and ventral midbrain. Brain Mn, iron, Zn, and copper concentrations were measured in 5-9 month mice. The results show Mn administration increased Mn concentrations in the brain and levels were higher in Mn-treated mutants than Mn-treated WT mice. Mn enhanced sensorimotor function in 13a2 mice compared to saline-treated mutants. Autofluorescence was significantly increased in the substantia nigra of older Mn-treated compared to saline-treated mutants. Furthermore, insoluble aSyn protein levels were significantly increased in the ventral midbrain but not in the dorsal midbrain in Mn-treated compared to saline-treated mutants. These results indicate loss of ATP13A2 function leads to increased sensitivity to Mn *in vivo*.

2357 Manganese Toxicity in *C. elegans*: Role of Insulin/Insulin-Like Growth Factor-1 Signaling Pathway (IIS)

T. Vieira Peres[3], L. Arantes[2], M. R. Miah[3], R. B. Leal[1] and M. Aschner[3]. [1]*Departamento de Bioquímica, Universidade Federal de Santa Catarina, Florianópolis, Brazil;* [2]*Departamento de Bioquímica, Universidade Federal de Santa Maria, Santa Maria, Brazil and* [3]*Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.*

Manganese (Mn) is an essential metal, but in excess it may cause a syndrome similar to Parkinson's disease, called manganism. In the model organism *Caenorhabditis elegans* Mn has been shown to induce dopaminergic degeneration, oxidative stress and increased levels of AKT. The evolutionarily conserved insulin/insulin-like growth factor-1 signaling pathway (IIS) participates in worm longevity, metabolism and antioxidant responses by antagonizing the transcription factors DAF-16/FOXO and SKN-1/Nrf-2. To study the participation of IIS in *C. elegans* response to Mn intoxication, wild-type (N2) and loss-of-function mutant (for proteins of the IIS) worms were exposed to Mn (2.5 to 100 mM) for 1h at the L1 larval stage. Strains with loss of function in akt-1, akt-2 and sgk-1 had higher resistance to Mn compared to N2 in the survival test. These strains also presented increased DAF-16 nuclear localization compared to N2. qRT-PCR data indicate increased expression of SKN-1 in akt-2 mutants and GCS-1 antioxidant enzyme in akt-1 mutants. Notably, the expression of sod-3 (homologue of superoxide dismutase) was increased in the akt-1 mutant worms independent of Mn treatment. However dopaminergic neurons similarly degenerated even in the more resistant strains. Dopaminergic function was evaluated with the basal slowing response behavioral test and dopaminergic neurons integrity was evaluated using worms expressing green fluorescent protein (GFP) under the dopamine transporter (DAT-1) promoter. These results suggest that IIS networks and their target proteins DAF-16 and SKN-1 may play a role in *C. elegans* response to Mn intoxication. However, this effect is not present in the dopaminergic neurons. This study demonstrates the importance of intracellular signaling pathways to the antioxidant response induced by Mn and features the IIS as an important point of research into the mechanisms of toxicity induced by Mn. Supported by NIH ES R0110563; CNPq.

2358 Combined Exposure to Methyl Mercury and Manganese During L1 Larvae Stage Causes Motor Decrease, Cholinergic Up Regulation and Oxidative Stress in L4 *Caenorhabditis elegans*

M. R. Schetinger[1,3], L. Arantes[1,3], T. V. Peres[3], A. B. Bowman[2] and M. Aschner[3]. [1]*Federal University of Santa Maria, Santa Maria, Brazil;* [2]*Department of Neurology, Vanderbilt University Medical Center, Nashville, TN and* [3]*Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.* Sponsor: M. Aschner.

Humans are exposed simultaneously to a variety of neurotoxic agents, including methylmercury (MeHg) and manganese (Mn). There is a potential pandemic caused by environmental contaminants with unpredictable long-lasting neurobehavioral sequelae. Therefore, the study of the combined exposures to toxicants is timely. The goals of this work were: 1) to study changes in the cholinergic system, focusing on acetylcholinesterase (ACE-2) and choline-acetyltransferase (CAT-1) expression; 2) to study the expression of the enzymatic anti-oxidant markers superoxide dismutase (SOD-3 and SOD-4) and catalase (CTL-1); and 3) to evaluate cholinergic neurodegeneration. *C. elegans* in the L1 larvae stage were exposed to Mn (1-100mM) or MeHg (1-60μM). All analyses were done in the L4 larvae stage in wild-type N2 or LX-929 (green fluorescent protein (GFP) expressed in all cholinergic neurons) strains. LD50 for Mn and MeHg were 17mM and 30μM, respectively. Based on the LD50, three exposure combinations were chosen: 10mM Mn+10μM MeHg, 20mM Mn+20μM MeHg and 50mM Mn+50μM MeHg. It was observed that the number of body bends and pharyngeal pumping were diminished in worms exposed to 20mM+20μM or 50 mM+50 μM. Total brood egg numbers were diminished only in the 50mM+50μM group. Relative mRNA content of ACE-2, CAT-1, SOD3, SOD4 and CTL-1 was increased in the 50 mM+50μM group. Cholinergic degeneration was observed in all groups. These changes in the cholinergic and enzymatic anti-oxidant systems might be related to the toxic effects of these metals. Finally, it was observed that the combined exposure to metals was more harmful than when exposed to a single metal alone.

2359 Microglia Amplify Neuroinflammatory Activation of Astrocytes During Exposure to Manganese

R. Tjalkens and K. Kirkley. *Center for Environmental Medicine, Colorado State University, Fort Collins, CO.*

Manganism or manganese neurotoxicity is a disease caused by chronic exposure to elevated levels of the essential trace metal manganese (Mn) that results in clinical motor deficits reminiscent of PD attributed to neuropathology within the basal ganglia of affected individuals. The pathogenetic events that lead to neuronal loss in this disease are largely undefined; however, recent literature has shown a prominent presence of neuroinflammation with increased expression of proinflammatory genes such as TNFα, IL-1β and nitric oxide synthase 2 (NOS2) in rodent and non-human primates treated with Mn. We postulated that during Mn exposure, microglia transition from a resting to activated phenotype prior to overt neuropathology and release pro-inflammatory factors such as TNFα and IL-1β that can act on astrocytes to induce secondary inflammatory responses. To test this hypothesis, we exposed immunopurified cultures of microglia isolated from neonatal mouse brain to increasing concentrations of Mn (0, 10, 30, or 100μM MnCl2) for 24 hr, resulting in dose-dependent increases in mRNA levels of Nos2, Il6, Il-1β, Tnfα, and caspase 1. Activated microglia displayed a mixed M1 phenotype, consistent with upregulation of CD86 and downregulation of CD32 and CD16. Following exposure to Mn, microglial-conditioned medium (MCM) resulted in dramatic upregulation of pro-inflammatory gene expression in primary astrocytes, as did Mn exposure with co-cultured microglia and astrocytes. RNAi for TNFα in primary microglia ablated these effects, demonstrating the requirement for this factor in microglia-dependent astrocyte activation. Moreoever, general inhibition of the NF-kappaB activating kinase, IKK, in microglia similarly inhibited Mn-mediated activation of astrocytes. Taken together, these results suggest that Mn directly stimulates inflammatory gene expression in microglia that subsequently promotes sustained activation of astrocytes. These cell-cell interactions are likely critical to neuroinflammation during exposure to Mn. This work was supported by a grant from the Michael J. Fox Foundation (RBT) and by NIH-ES021656 (RBT).

2360 Manganese Inhibition and Activation of Mitochondrial Oxidative Processes in Neuronal Cells

J. Fernandes, L. Hao, K. M. Bijli, J. D. Chandler, M. Orr, D. P. Jones and Y.-M. Go. *Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Emory University School of Medicine, Atlanta, GA.*

Manganese (Mn) is the second most prevalent transition metal and occurs naturally in food, drinking water and air at low doses. Mn is an essential nutrient required for growth, but excessive exposure at higher concentrations causes neurotoxicity via oxidative stress to mitochondria. However, the impact of low dose of Mn, as obtained from food and water, on mitochondrial metabolism has not been established. In the present study, we investigated dose-dependent effects of Mn on mitochondrial oxidant production and cellular bioenergetics in SH-SY5Y neuroblastoma cell line. Cells were treated for 5 hours with MnCl2 concentrations (0, 1, 5, 10, 50, 100 μM), a range presenting normal through toxic conditions. Our data show that Mn treatment leads to a dose-dependent increase in cellular Mn uptake [2 fold increase (5 μM Mn), 7 fold increase (100 μM Mn)]. The redox states (redox potential, Eh) of the extracellular and intracellular redox couples GSH/GSSG and cysteine/cystine (Cys/CySS) represents an oxidized environment with Mn treatment accompanied by a dose dependent increase in mitochondrial oxidant levels [25% increase (5 μM Mn), 50% increase (100 μM Mn)]. Basal oxygen consumption rate decreased with increasing Mn concentration [33% decrease (5 μM Mn), 66% decrease (100 μM Mn)]. Taken together, our data show that SH-SY5Y cells respond to Mn from low to high concentrations in a dose-dependent manner, which enables us to monitor mitochondrial functional changes systematically. The present results show that Mn has different dose-response effects on mitochondria, with low doses causing respiratory inhibition and higher doses increasing oxidant production. These results provide critical information to further investigation on measuring integrative mitochondrial and cellular signaling networks associated with environmental stress such as Mn concentration.

2361 Valproic Acid (VPA) Attenuates Manganese-Induced Repression of Astrocytic Glutamate Transporters Promoter Activities and Neurological Behavioral Deficits in Mice

J. A. Johnson[2], P. Karki[2], K. Smith[2], D.-S. Son[2], M. Aschner[1] and E. Lee[2]. [1]*Physiology, Albert Einstein School of Medicine, New York, NY and* [2]*Physiology, Meharry Medical College, Nashville, TN.*

Chronic exposure to elevated levels of manganese (Mn) has been shown to cause neurological disorders with shared features of Parkinson's Disease (PD), referred to as manganism. The mechanism(s) of Mn-induced neurotoxicity remains to be established, but previous studies have reported that Mn overexposure decreases the expression as well as function of astrocytic glutamate transporters, glutamate-aspartate transporters (GLAST) and glutamate transporter-1 (GLT-1), which are directly associated with excitotoxic neuronal injury. Additionally, epigenetic modifiers histone deacetylases (HDACs) and yin yang 1 serve as co-repressors of glutamate transporters GLAST and GLT-1 promoters in the presence of elevated levels of manganese. In this study, valproic acid (VPA), an HDAC inhibitor, is used to test if it attenuates Mn-induced down-regulation of GLAST/GLT-1 promoter activity *in vivo* as well as it protects Mn-induced neurological behavioral deficits in mice. We used double transgenic fluorescent reporter mice induce the expression of enhanced green fluorescent protein (eGFP) for GLT-1 and Discosoma sp. Red fluorescent protein (dsRed) for GLAST promoter activities. Mice were exposed daily with 200mg/kg of VPA or saline intraperitoneal pretreatment followed by intranasal instillation of 30mg/kg of MnCl2 or ddH2O for two weeks prior to open field and rotorrod behavior tests and confocal imaging of brain sections. Results showed that VPA pretreatment attenuated Mn-induced decrease of locomotor activity and motor coordination respectively. Additionally, confocal imaging showed that VPA pretreatment attenuated Mn-induced decreases of GLT-1 and GLAST promoter activities in the striatum and midbrain regions of the mouse brain. These findings suggest that VPA could be developed as a potential neuroprotectant against Mn-induced neurological disorders since it prevents Mn-induced neurological behavioral deficits and also attenuates Mn-induced repression of astrocytic glutamate transporters.

2362 Pre- and Postnatal Exposure to Manganese Induces Dysregulation of Glutamate Transporter 1(GLT-1) and Adult Neurogenesis via the Transcription Factor Yin Yang 1 (YY1) in Mice

K. Smith[2], P. Karki[2], M. Aschner[1] and E. Lee[2]. [1]*Albert Einstein School of Medicine, New York, NY and* [2]*Neuroscience & Pharmacology, Meharry Medical College, Nashville, TN;* [3]*Physiology, Meharry Medical College, Nashville, TN.*

Chronic exposure to high levels of manganese (Mn) from environmental or occupational sources causes manganism, a neurodegenerative disorder that displays features similar to Parkinson's disease (PD). The mechanism of Mn-induced neurodegeneration is not completely understood, but Mn decreases the expression and function of the astrocytic glutamate transporter 1 (GLT-1), which is responsible for removing excess glutamate from extracellular spaces and its dysregulation has been linked to a number of neurodegenerative disorders, including PD. Interestingly, excessive exposure to Mn has been linked to motor impairment and deficits in neurodevelopment and intellectual functions in developing children. However, the mechanism by which Mn disrupts neurodevelopment has yet to be established. Previously, we found that the transcription factor Yin Yang 1 (YY1) plays a critical role in mediating Mn-induced repression of GLT-1. To further investigate the role of YY1 in Mn-induced repression of GLT-1, we generated an astrocytic-specific conditional YY1 knockout (YY1cKO) mice using the Cre/loxP system. In this study, YY1cKO mice exposed to Mn via drinking water from embryonic day 10 through postnatal day 21 showed attenuation in the Mn-induced reduction in the expression of GLT-1 and tyrosine hydroxylase as confirmed by western blotting and immunohistochemistry. Moreover, Mn is known to dysregulate adult neurogenesis leading to learning and memory dysfunction. We used LUHMES cells, which are dopaminergic stem cells, and found that Mn decreased neuronal differentiation. Combined, our studies show that YY1 is a critical regulator of Mn induced neurotoxicity and that Mn inhibits neuronal differentiation. Supported by NIEHS R01 ES024756.

2363 Increased Interactive Toxicities on the Blood-CSF-Barrier (BCB) Following Co-Exposure to Manganese (Mn) and Lead (Pb), *In Vitro*

D. Cholger, X. Fu and W. Zheng. *Health and Human Sciences, Purdue University, West Lafayette, IN.*

Co-exposure to Mn and Pb takes place in occupational as well as environmental settings. Mn is known to cause a movement disorder called manganism, while Pb induces neurological dysfunction and has been suspected to be associated with Alzheimer's disease. Neurotoxicities produced by each metal alone have been extensively documented; yet the combined toxicity as often occurs in real life was virtually unknown. This study was designed to test the hypothesis that Mn and Pb co-exposure may cause more damage to the BCB in the choroid plexus than either metal alone. An immortalized Z310 cell-based BCB model was chosen, for the choroid plexus is known to harbor both metals and has the ability to transport materials in and out of the brain. Our data showed that exposure to Mn 250 µM or Pb 10 µM caused 8.7% ($p<0.05$) or 37.0% ($P<0.001$) cell death by MTT assay, respectively. In contrast, co-exposure to Mn and Pb showed a marked increase of cell death by 73.5%, which was higher than those exposed to individual metal ($p<0.001$). At Mn exposures <100 µM, it was noted that Mn had a protective effect against Pb-induced cytotoxicity until the Mn concentration surpassed 100 µM. Atomic Absorption Spectroscopy showed an increased intracellular accumulation of both Mn and Pb in co-exposed groups versus either metal alone ($p < 0.05$; $p < 0.001$ respectively). Since increased metal accumulation could be due to an increase in expression of divalent metal transporter 1 (Dmt1) and/or copper transporter (Ctr1), we determined the expression level of mRNAs encoding Dmt1 and Ctr1. Our qPCR data suggested that the expression of both Dmt1 and Ctr1 was increased in co-exposed groups compared to single exposed groups ($p < 0.01$). These data provide the first-hand evidence that the interactive toxicity following co-exposure to Mn and Pb is greater than the toxicity caused by exposure to each metal alone. These findings may have clinical implications to workers engaging in smelting, welding and mining productions. (Supported in part by NIEHS Grant ES008146)

2364 Characterization of Intracellular Mn-Modifying Small Molecules Identified in a High Throughput Screen

K. J. Horning[2], K. K. Kumar[2], M. Aschner[1] and A. B. Bowman[2]. [1]*Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY and* [2]*Neurology, Vanderbilt University Medical Center, Nashville, TN.*

Manganese (Mn) is a toxic and redox-active metal that is also an essential cofactor for life. Disruption of Mn biology has been implicated in the pathogenesis of Parkinson's disease and Huntington's disease. Though regulation of Mn transport has been well described on a systemic level, mechanisms of its transport and handling within cells are unclear. Recently, 41small molecules were identified by a high throughput screen that increase or decrease intracellular Mn in a concentration-dependent manner, using a mouse striatal neuron line, deriving from a brain region that selectively accumulates Mn at basal and pathogenic conditions. The 39 of the currently available compounds - our "Mn toolbox" - can be used as chemical biology tools to study Mn regulation at the cellular level. We report that all 39 small molecules tested manipulate Mn via mechanisms independent of the SV40 Large T-antigen - which has been previously shown to influence Mn uptake. We report that none of these small molecules significantly chelate Mn in aqueous media. Of the 39 twenty small molecules retained activity in media over 18 hours with 50µM Mn and showed minimal toxicity (< 25% cell death). Interestingly, a portion of the "Mn toolbox" displayed genotype-dependent activity differences between the wild-type (WT) striatal cells used in the screen, and those expressing mutant Huntingtin. Mutant huntingtin-expressing cells display a deficit in net Mn uptake following exposure, though this phenotype was suppressed, reversed, or exaggerated following small molecule co-treatment. However, only four of the 39 compounds tested in HeLa cells had significantly different activities in Mn handling ($p < 0.05$), compared to their activity seen in WT striatal cells. These four small molecules presented a decreased effect size in HeLa cells versus WT striatal neurons. The results suggest neural and non-neural cells have similar mechanisms of Mn-handling that may be altered by pathogenic mutations in Huntingtin. Supported by ES010563 (MA and ABB) and ES016931 (ABB)

2365 BTBD9, a Restless Leg Syndrome Associated Protein, Regulates Manganese-Induced Toxicity in *C. elegans*

P. Chen[2], J. Bornhorst[1], K. H. Lee[2], A. B. Bowman[3] and M. Aschner[2]. [1]*Food Chemistry, Institute of Nutritional Sciences, University of Potsdam, Nuthetal, Germany;* [2]*Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY and* [3]*Neurology, Vanderbilt University, Nashville, TN.* Sponsor: M. Aschner.

Restless Legs Syndrome (RLS) is a common neurological disorders seen in ~10% of the US population. RLS-associated sleep deprivation can seriously impact life quality, causing anxiety, depression and attention-deficit/hyperactivity disorder (ADHD) symptoms. Moreover, RLS may portend hypertension, heart disease and stroke. RLS exhibits both familial and non-familiar (idiopathic) forms, with ~60% of cases having a family history of the disease. BTBD9 is one of the genetic risk factors, associated with decreased serum iron (Fe) level. Interestingly, lymphocytes from RLS patients have an altered Fe management protein profile, which also regulates manganese (Mn) homeostasis. This raises the question as to whether the symptoms inherent to RLS patients are the result of Fe deficiency or elevated concentrations of another metal that opportunistically increases when Fe levels are low. Here we present novel data that BTBD9 functions to regulate Mn homeostasis in Caenorhabditis elegans (*C. elegans*). A blast search identified hpo-9 as the BTBD9 homolog in *C. elegans*, with ~75% sequence similarity. A mutant strain (tm3719) carrying 761 bp deletion of hpo-9 was obtained. We found that tm3719 (hpo-9-/-) worms were more sensitive to Mn exposure. In the absence of Mn, hpo-9-/- animals grew normally. At 10 and 25 mM of Mn treatment, the survival rates of hpo-9-/- worms were significantly lower than wild type (WT) worms. Mn concentrations in hpo-9-/- worms were higher than WT worms. Meanwhile, hpo-9 expression level was increased at low concentration (0.1 mM) of Mn exposure, but decreased at high concentration (2.5 mM). To better characterize HPO-9 protein, a transcriptional fusion construct was created with green fluorescent protein (GFP) driven under hpo-9 promoter (~1.5 kb upstream of hpo-9 start codon). We found that GFP was present high in the head and pharynx and low in the intestine and seam cells. Using a confocal microscopy and co-localization study, we found that hpo-9 was expressed in dopaminergic neurons, indicating that HPO-9 might play a role in dopamine signaling. Together, our results suggest a novel role for hpo-9/BTBD9 in regulating Mn homeostasis and possibly dopamine signaling in *C. elegans* (supported by NIEHS ES010563).

2366 Aberrant Adult Neurogenesis in the Subventricular Zone (SVZ)-Rostral Migratory Stream (RMS)-Olfactory Bulb (OB) System Following Subchronic Manganese (Mn) Exposure

X. Fu[1], W. Jiang[1], X. Gao[2], A. Zeng[1], D. Cholger[1], J. Cannon[1], J. Chen[2] and W. Zheng[1]. [1]*School of Health Sciences, Purdue University, West Lafayette, IN and* [2]*School of Medicine, Indiana University, Indianapolis, IN.* Sponsor: W. Zheng.

Adult neurogenesis occurs in the SVZ of brain lateral ventricle; newborn neuroblasts migrate along RMS, providing the cells to the OB and nearby brain structure. Our recent studies reveal a significant reduction of Cu content and increased expressions of DMT1 and BrdU in Mn-exposed SVZ. This study was designed to further determine whether Mn exposure disturbed the proliferation, migration, survival and differentiation of neuroblasts from SVZ via RMS to OB. Adult rats received a single dose of BrdU after 4-wk Mn exposure to label the proliferating cells in SVZ. Immunostaining data showed a total of (89.9±17.5)×103 BrdU(+) cells in Mn-exposed SVZ, which was 48% higher than that in the control (60.6±2.4)×103 ($p<0.05$). The BrdU(+) proliferating cells were identified as a mixed population of GFAP-, Nestin- and DCX-positive cells. To investigate how these newborn cells migrated, survived and differentiated, adult rats received BrdU injections for 3 consecutive days prior to Mn exposure. The fates of BrdU-labeled cells in the RMS and OB were traced during and after Mn treatment. After 2-wk Mn exposure, 95% of the BrdU(+) cells were found to migrate away from the SVZ and RMS, and mainly distribute in the granular cell layer (GCL) and mitral cell layer (MCL) of OB. Mn exposure significantly reduced the number of surviving BrdU/DCX/DAPI-labeled immature neurons in the OB (49% survival rate) as compared to the control (92% survival rate) ($p<0.01$). At the end of 4-wk treatment, most of the surviving BrdU(+) cells within the GCL and MCL were differentiated into NeuN(+) mature neurons. However, survival rates of BrdU/NeuN/DAPI-labeled cells in OB were 33% and 64% in the 4-wk Mn-exposed and control animals, respectively ($p<0.01$). To study whether Cu had a direct effect on the cell proliferation in adult SVZ, an i.c.v. injection was used to deliver Cu into the lateral ventricle. Our data showed that high levels of Cu in the CSF decreased BrdU(+)

proliferating cells in SVZ. Taken together, these results suggest that Mn exposure enhances the cell proliferation in adult SVZ, but significantly reduces the surviving adult-born cells and markedly inhibits their differentiation into mature neurons, resulting in an overall decreased adult neurogenesis in the OB. (Supported by NIEHS ES008146)

PS 2367 Decreased Fractional Anisotropy in Globus Pallidus of "Asymptomatic" Welders

E. Lee[5], M. R. Flynn[1], G. Du[5], M. M. Lewis[5], A. H. Herring[1], L. Kong[5], R. B. Mainman[5] and X. Huang[5]. *[1]Biostatistics, University of North Carolina, Chapel Hill, NC; [2]Environmental Sciences & Engineering, University of North Carolina, Chapel Hill, NC; [3]Neurology, Pennsylvania State University College of Medicine, Hershey, PA; [4]Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA and [5]Public Health Sciences, Pennsylvania State University College of Medicine, Hershey, PA.*

Introduction: Welding fumes contain several metals including manganese (Mn), iron (Fe), and copper (CU), which at high exposure may be neurotoxic. The relationship between metal accumulation and brain pathology, however, is unclear. This study examined the microstructural changes associated with chronic welding exposure in "asymptomatic" welders. Method: Subjects with (n=43) and without (controls; n=31) history of welding were studied. Occupational questionnaires estimated recent (HrsW; hours of welding, past 90 days) and lifetime welding exposure (YrsW; years welding, life time). Whole blood metal levels (Mn, Fe and Cu), brain MRI R1 (1/T1) and pallidal index (PI; both to gauge Mn), and R2* (1/T2*; to gauge Fe) were obtained to estimate welding-related metal accumulation. Diffusion tensor imaging (DTI) measures were used to assess microstructural changes in basal ganglia [caudate, putamen, and globus pallidus (GP)] and correlated with exposure measurements. Results: Compared to controls, welders had significantly higher blood Mn, Fe and Cu levels (ps<0.001) and lower fractional anisotropy (FA) in the GP [p= 0.011 after multiple comparison correction]. The decreased GP FA was also evident for welders who had not welded for at least 5 months prior to the MRI acquisition (p=0.042). GP FA values were associated with YrsW for welders with higher recent exposure (R=-0.68, p=0.004), but not HrsW or blood metal levels (ps >0.08). Within welders, GP FA was negatively associated with PI (R=-0.46, p=0.002), but not with R1 or R2* values (ps >0.12). Conclusion: This study demonstrated for the first time that GP FA may capture microstructural damage that may be associated with long term welding exposure in "asymptomatic" welders. The decreased GP FA may be related to Mn accumulation. Future studies are needed to replicate the findings and the neurobehavioral relevance.

PS 2368 Developmental Manganese Overexposure or Adult 6-hydroxydopamine Striatal Lesions Induce Exacerbated Learning Deficits in Combination

R. A. Bailey[2], A. Gutierrez[2], R. M. Amos-Kroohs[2], C. V. Vorhees[1,2] and M. T. Williams[1,2]. *[1]Department of Pediatrics, University of Cincinnati, Cincinnati, OH and [2]Division of Neurology, Cincinnati Children's Research Foundation, Cincinnati, OH.*

Neurodegenerative disorders are on the rise and a number of environmental factors have been suggested to predispose or exacerbate these risk factors. Manganese (Mn) is an abundant element and essential for human growth and development, however Mn pollution from sources such as gasoline additives, steel mills, and some well-water may adversely affect neurodevelopment. In adults, manganese overexposure (MnOE) can cause manganism, which has some similarities to Parkinson's disease in terms of behavioral, cellular, and genetic changes. In humans and animals, MnOE may predispose to Parkinson's disease or cause Parkinson-like effects. We gavaged male and female rats with MnCl (100 mg/kg, expressed as free metal) or a NaCl vehicle from postnatal day (P)4 to P28 every other day. This regimen produces increased blood Mn levels similar to those found in children in Mn polluted regions. As adults, rats were injected with saline or 6-hydroxydopamine (6-OHDA) into the dorsal striatum at concentrations that deplete dopamine, but do not induce motor deficits. After recovery, animals were tested for motor effects in an open-field, for egocentric learning in the Cincinnati water maze (CWM), spatial learning in the Morris water maze, and in fear conditioning. Animals that were exposed to Mn or 6-OHDA alone had deficits in egocentric learning that were exacerbated in combination. In the Morris water maze, the deficits were not as pronounced, but showed a similar pattern. In the open-field, animals showed no motor deficits; conditioned fear data are still being analyzed. Overall, the data suggest that MnOE may be a risk factor when exposure occurs during development for Parkinsonism later in life. (Supported by NIH T32 ES07051).

PS 2369 Mitochondrial DNA Damage and Dysfunction and Their Effects on Dopaminergic Neurodegeneration after Toxic Insult in *pink-1* and *pdr-1* Mutant Strains of *Caenorhabditis elegans*

C. P. Gonzalez-Hunt, I. T. Ryde and J. N. Meyer. *Nicholas School of the Environment, Duke University, Durham, NC.*

The environmental components of Parkinson's disease (PD) etiology have received more attention in recent years, as it is increasingly clear that genetic factors alone cannot explain most cases of the disease. Exposure to known or suspected mitotoxicants has been linked to PD, and mitochondrial dysfunction itself has been associated with dopaminergic neurodegeneration. We investigated dopaminergic neuron viability after toxic insult in Caenorhabditis elegans strains with gene knockouts of pink-1 and pdr-1, which are genes involved in mitochondrial dynamics. These genes are the nematode homologs of the human genes PINK1 and PARK2 which when mutated cause familial PD. The four cephalic dopaminergic neurons were visualized with fluorescent microscopy, and assigned a score based on the level of damage observed. Each neuron was scored from 0 to 2, with zero representing an intact dendrite and 2 representing the highest level of neurodegeneration observed. The strain BY200 (dat-1::GFP) was used as wild type for the strains UA226 (dat-1::GFP; pink-1) and UA227 (dat-1::GFP; pdr-1), all generously provided by Guy Caldwell (University of Alabama). After exposure to UV radiation C (a tool utilized to generate bulky adducts in mitochondrial DNA similar to those caused by polycyclic aromatic hydrocarbons), both mutant strains showed higher levels of neurodegeneration compared to wild type. After exposure to the oxidative agent 6-hydroxydopamine (6-OHDA), the pdr-1 mutant again showed higher levels of neurodegeneration compared to wild type, but the pink-1 mutant exhibited less neuronal damage than wild type. Currently, studies are being performed to investigate the role of the antioxidant defenses in protecting dopaminergic neurons from toxicant-induced degeneration, particularly in the context of the mitochondrial mutant strain pink-1.

PS 2370 The Selective mGluR Group I Agonist, 2-chloro-5-hydroxyphenylglycine, Increases BDNF and Myelin Proteins Following Cuprizone-Induced Demyelination

K. S. Saitta[1,2], Y. Huang[1] and C. F. Dreyfus[1]. *[1]Department of Neuroscience and Cell Biology, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ and [2]Department of Pharmacology & Toxicology and Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.*

Brain-derived neurotrophic factor (BDNF) is an important neurotrophin involved in the development and survival of oligodendrocyte lineage cells. However, the role of endogenous BDNF in demyelinating diseases such as multiple sclerosis is not well understood. In fact, patients with relapsing-remitting multiple sclerosis have decreased levels of BDNF. Therefore, an intriguing therapeutic approach for these types of demyelinating diseases may be to enhance the endogenous source of BDNF. We have previously demonstrated that the Group I and Group II mGluR agonist, ACPD, was able to enhance the expression of BDNF and myelin proteins when injected directly into the corpus callosum of mice following a cuprizone-induced lesion. The aim of this study was to expand this concept to the selective mGluR Group I agonist, 2-chloro-5-hydroxyphenylglycine (CHPG), by using the more clinically relevant approach of a peripheral injection. Male C57BL/6 mice were fed either 0.2% cuprizone milled into mouse feed or identically processed control feed for 4 weeks starting at 8 weeks of age. Cuprizone is a copper chelator used to induce demyelination in the corpus callosum. CHPG or saline vehicle was injected intraperitoneally after 4 weeks of continuous treatment, and the levels of BDNF, myelin proteins, and progenitor cell markers were analyzed by Western blot. 4 weeks of cuprizone treatment caused a decrease in BDNF and myelin proteins within the corpus callosum. Cuprizone also increased oligodendrocyte progenitor cell markers NG2 and PDGFRα compared to mice on control feed. However, intraperitoneal injections of CHPG (20 or 40 mg/kg) to the curprizone-treated mice reversed cuprizone effects and increased levels of BDNF and myelin proteins while also decreasing oligodendrocyte progenitor markers. Importantly, CHPG did not alter these proteins when given to mice receiving control feed. Taken together, these data suggest that selective mGluR Group I agonists such as CHPG may be a therapeutic approach for treating demyelinating diseases by increasing the levels of BDNF and myelin proteins possibly by promoting differentiation of oligodendrocyte progenitor cells. (Supp: NIH NS036647; HD023315; T32ES007148 and NMSS RG 4257B4/1)

2371 MDR1 Transporter Protects Against Paraquat-Induced Dopaminergic Neurodegeneration

D. You[3], C. J. Gibson[3], M. M. Hossain[1], X. Wen[3], I. Yang[3], B. Buckley[3], J. R. Richardson[1] and L. M. Aleksunes[3]. *[1]Northeast Ohio Medical University, Rootstown, OH; [2]Department of Pharmacology and Toxicology, Rutgers, the State University of New Jersey, Piscataway, NJ and [3]Environmental and Occupational Health Sciences Institute, Rutgers, the State University of New Jersey, Piscataway, NJ; [4]Joint Graduate Program in Toxicology, Rutgers, the State University of New Jersey, Piscataway, NJ.*

Prior studies have observed a loss-of-function genetic polymorphism and overall reduction in the expression and function of the multidrug resistance protein 1 (MDR1, ABCB1) transporter in Parkinson's disease patients, primarily in those exposed to pesticides such as paraquat. Therefore, MDR1 may be a key regulator of gene-environment interactions in PD. We sought to determine the role of MDR1 in the transport of paraquat and determine whether loss of Mdr1 function in mice altered susceptibility to neurotoxicity. In human brain capillary endothelial (hCMEC/D3) cells, reduction of MDR1 transport activity using the inhibitor PSC833 or siRNA knockdown increased intracellular accumulation of paraquat by 200%. *In vivo* studies assessed the toxicity of paraquat in the midbrains of mice lacking the Mdr1a/1b transporters. Knockout mice had increased susceptibility to paraquat-induced neurotoxicity. One week after a single dose of paraquat (10 mg/kg i.p.), Mdr1a/1b knockout mice had a 40% reduction in TH+ dopaminergic neurons in the substantia nigra pars compacta compared to wild-type mice, which did not exhibit significant reductions. Mdr1a/1b knockout mice treated with paraquat also exhibited a more profound reduction in the expression of the dopamine transporter (DAT) and increased levels of alpha-synuclein in the midbrain. Staining of DAT and OCT3, two transporters responsible for the entry of paraquat into cells, revealed similar baseline expression in the two genotypes, suggesting that the effect is specific for Mdr1. Collectively, these data demonstrate that the MDR1 transporter plays an important role in the efflux of paraquat and protects against paraquat-induced neurotoxicity. Funded by R01ES021800, T32ES007148, P30ES005022 and an AFPE Fellowship.

2372 Mechanisms of PhIP-Induced Dopaminergic Neurotoxicity

Z. S. Agim[2], A. Jacquet[2], J.-C. Rochet[2] and J. R. Cannon[2]. *[1]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN and [2]School of Health Sciences, Purdue University, West Lafayette, IN.*

Parkinson's disease (PD) is the second most common neurodegenerative disease. While many brain regions are involved, it is the loss of nigral dopamine neurons that produces the majority of cardinal motor symptoms. Environmental factors have been repeatedly linked to PD etiology and dopaminergic neurotoxicity. The current studies were aimed at testing whether dietary toxicants selectively lesion dopaminergic neurons and at identifying mechanisms of action. 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) is a heterocyclic amine, produced in high temperature cooked meat. It is classified as a Group 2B carcinogen and primarily investigated as a mutagen. However, our data in primary midbrain cultures has shown that PhIP and its Phase I metabolite, N-OH-PhIP, are selectively neurotoxic to dopaminergic neurons. Here, we aimed to test mechanisms of PhIP-induced dopaminergic neurotoxicity *in vitro*. We tested whether PhIP treatment elicited Nrf2 activation, proteasome inhibition and interaction with cytosolic dopamine pools *in vitro*. PhIP treatment at three different concentrations, 0.1, 1 and 10 μM, did not induce Nrf2 activation or proteasome inhibition in primary midbrain cultures. However, a nontoxic dose of PhIP (200 nM) induced dopaminergic toxicity in primary cultures when co-treated with reserpine, an inhibitor of the vesicular monoamine transporter VMAT2. VMAT2 inhibition is expected to increase levels of free, reactive cytosolic dopamine. Such conditions have been shown to exacerbate toxicity of known dopaminergic neurotoxicants. Thus, a role for VMAT2 and cytosolic dopamine in mediating PhIP neurotoxicity warrants further investigation. We also investigated PhIP neurotoxicity *in vivo*. Rats treated with acute doses (100 or 200 mg/kg) for 8 or 24 hours exhibited increased nitrotyrosine levels in dopaminergic neurons of the substantia nigra. Immunohistochemical analysis did not show overt evidence of glial activation, but dopaminergic neurons showed an abnormal morphology. These findings suggest that PhIP may also result in selective neurotoxicity *in vivo*. Our future studies will continue to focus on mechanisms of PhIP-induced neurotoxicity.

2373 c-Abl Regulates Rotenone-induced Inflammatory Response via the Activation of NLRP3 Inflammasome

V. J. Lawana, N. Singh, S. Sarkar, H. Jin, V. Anantharam, A. Kanthasamy and A. Kanthasamy. *Department of Biomedical Sciences, Iowa State University, Ames, IA; Interdepartmental Toxicology Program, Iowa State University, Ames, IA.*

Multiple evidences support the hypothesis that exposure to pesticides increases the risk of PD. Emerging evidence indicates that intracellular inflammasome complex namely NLRP3 complex is involved in the recognition and execution of host inflammatory response. Thus in the present study we investigated the hypothesis that NLRP3 inflammasome activation is linked to rotenone-induced microglial activation which is dependent upon a priming stimulus by a pathogen associated molecular pattern (PAMP) or damage associated molecular pattern (DAMP) respectively. We employed primary microglia, BV-2 microglial cell culture, and an *in vivo* endotoxin model of neurodegeneration to address the stated hypothesis. We found that LPS priming accelerated rotenone-induced NLRP3 inflammasome activation that was associated with the activation of caspase-1 and subsequent proteolytic processing and release of IL-18 and IL1β as well as the release of TNFα and IL-6 as assessed via WB analysis and Luminex multiplex technology. Mechanistic studies revealed c-Abl/PKCδ kinase signaling axis as a proximal signal that exacerbated rotenone-induced NLRP3 inflammasome activation, that is mediated via mitochondrial and autophagolysosomal system (ALS) dysfunction and accompanying downregulation of TFEB, a lysosomal transcription factor. Intriguingly, gene silencing and pharmacological inhibition of c-Abl attenuated NLRP3 inflammasome activation, mitochondrial and ALS dysfunction and PKCδ activation; while, c-Abl overexpression potentiated that response in LPS primed rotenone treated microglial cells. Furthermore, using an *in vivo* LPS model of neurodegeneration we showed that c-Abl upregulation positively correlated with NLRP3 inflammasome activation and accompanying sickness-like behavior. Our findings demonstrate for the first time that c-Abl/PKCδ signaling axis is a key regulator of NLRP3 inflammasome activation which is mediated partly via dysregulation of ALS and mitochondrial function during rotenone-induced microglial activation (supported by NS088206).

2374 Prokineticin 2 Plays a Role in Altered Neurogenesis and Non-Motor Deficits in PD Models

M. R. Langley, M. Neal, J. Luo, N. Kondru, C. Berg, H. Jin, V. Anantharam, A. Kanthasamy and A. Kanthasamy. *Interdepartmental Toxicology Graduate Program and Department of Biomedical Science, Iowa State University, Ames, IA.*

Recently, we showed that a secreted protein involved in olfactory bulb neurogenesis and circadian rhythms, prokineticin 2 (PK2), plays a novel compensatory protective role in nigral dopaminergic neurodegeneration in Parkinson's disease (PD), yet the exact mechanism of the neuroprotective response remains elusive. We hypothesize that PK2 potentially rescues impaired neurogenesis in PD models by promoting differentiation, proliferation, or migration in the subventricular zone (SVZ). First, we observed that recombinant PK2 treatment of organotypic brain slices increased the number of BrdU+ cells in the SVZ, suggesting a stem cell proliferative action of PK2. Also, lentivirus-mediated overexpression of PK2 significantly increased Tuj1+ neurons in brain slices containing SVZ, providing evidence of increased differentiation. Next, we extended our studies to MitoPark mice, a transgenic mouse model of PD. Our previous studies have shown that these animals exhibit several clinically relevant non-motor symptoms including olfactory dysfunction, learning and memory deficits, and neuropsychological problems. Immunohistological staining for BrdU or Ki67 revealed less neurogenesis in the SVZ of aged MitoPark mice. Consistently, forced swim test (FST) increased neurogenesis in the hippocampus of control mice but not in MitoPark mice, as evidenced by increased CREB phosphorylation. However, antidepressant treatment of MitoPark mice restored CREB phosphorylation to littermate control levels, and resulted in the MitoPark mice spending significantly less time immobile during the FST. Importantly, PK2 transgene delivery by AAV in striatal regions enhanced neurogenesis in MitoPark mice and attenuated the corresponding non-motor symptoms at ages 16 and 24 wks as measured by the novel scent test and FST. Overall, these novel findings suggest that the development of non-motor symptoms in MitoPark mice coincides with impaired adult neurogenesis, and that PK2 may play a compensatory role in regulating neurogenesis by promoting proliferation and differentiation of neural stem cells (NIH NS78247, NS074443, ES19267 and Linda Lloyd Endowed Chair)

2375 Chronic Exposure to Manganese Causes Selective Changes in the Enteric Nervous System and Gut Physiology Leading to Altered Nutrient Metabolism and Intestinal Motility

S. Ghaisas, D. S. Harishchandra, H. Jin, V. Anantharam, A. Kanthasamy and A. Kanthasamy. *Interdepartmental Toxicology Graduate Program and Department of Biomedical Science, Iowa State University, Ames, IA.*

Despite evidence of gastrointestinal inflammation from exposure to manganese (Mn) in humans, the role of manganese toxicity in gastrointestinal abnormalities has not been studied in detail. Therefore, we evaluated the effect of manganese on the enteric nervous system using cell culture and animal models. First, Mn exposure to GFAP-positive enteric glial cells (EGCs) produced a dose-dependent toxicity, and an EC50 concentration was determined to be 8μM for over 24 hrs. The Mn toxicity in EGCs also revealed a dose-dependent increase in mitochondrial dysfunction as measured by MTS and MitoTracker assays accompanied by activation of the pro-apoptotic factor Bax. In contrast to EGCs, the enteric neuronal cell line and primary enteric cultures were more resistant to manganese toxicity (EC50 = 100 μM). 100 μM manganese treatment induced gene expression of proinflammatory factors, including iNOS, TNFα and IL-1β in primary enteric cells. Neuronal and glial toxicity were seen in Western blots as decreased expression of PGP9.5 and GFAP, respectively. Interestingly, chronic exposure to manganese by intragastric gavage for 30 days increased intestinal transit time, indicating impairment in gut motility. Mn-exposed mice also displayed intestinal inflammation evidenced by increased iNOS expression in the myenteric neurons of the duodenum. Evaluation of fecal metabolites by GC-MS revealed subtle disturbances in the carbohydrate metabolism early on in mice exposed to Mn. Specifically, increased levels of the palmitic acid and decreased levels of sedoheptulose were observed. Moreover, Periodic Acid Schiff-Alcian Blue staining of the duodenum and ileal sections revealed decreased mucopolysaccharide production in the goblet cells while western blot and slot blot revealed increased TNFα and aggregated proteins in the colons of these mice. Collectively, our data demonstrate for the first time that manganese exposure affects enteric neuronal and glial cell function and promotes an inflammatory response, contributing to altered gut microbiota and gastrointestinal motility. (Supported by NIH RO1 grants ES10586, ES19267 and Linda Lloyd Endowed Chair)

2376 Optineurin Expression in the Nigrostriatal Dopamine System in Normal and Rotenone-Treated Rats

J. Wise and J. Cannon. *Health Sciences, Purdue University, West Lafayette, IN.*

Parkinson's disease (PD) is a progressive neurodegenerative disease that affects ~5 million people around the world. Currently, the etiopathogenesis is poorly understood and curative or disease-modifying treatments are not available. Mechanistic studies have identified complex pathogenic pathways including: mitochondrial dysfunction, oxidative stress/damage, and protein aggregation. While the specific genes and proteins involved may vary, these pathways are commonly affected in many neurodegenerative diseases. Mutations in the protein optineurin (OPTN) have recently been identified as causative factors for glaucoma and amyotrophic lateral sclerosis. OPTN has multiple recognized roles in neurons, notably in mediating autophagic flux, which has been found to be disrupted in most neurodegenerative diseases. OPTN+ aggregates have been identified in cytoplasmic inclusions in numerous neurodegenerative diseases, including PD, but whether OPTN plays a role in PD pathogenesis has yet to be tested. Thus, we chose to test the hypothesis that dopaminergic neurotoxicants alter OPTN expression, leading to autophagy disruption and PD-relevant pathogenesis. To test our hypothesis, we characterized neuronal OPTN expression in normal animals and those treated with the mitochondrial toxicant rotenone, a pesticide commonly used to model PD. Our results show immunohistochemical evidence that OPTN is enriched in dopamine neurons in the midbrain, and its expression is modulated by rotenone treatment *in vivo*. Here, animals were sampled prior to overt neurodegeneration (early-stage) and after severe behavioral deficits (late-stage). The effect and magnitude of OPTN expression changes is dependent on duration of treatment. Furthermore, OPTN colocalizes with the autophagic vesicle marker LC3 in perinuclear puncta in late-stage rotenone-treated animals, potentially indicating an important role in autophagy. Future work will aim to determine how OPTN mediates PD-related autophagic dysfunction and whether modulation may possibly serve as a therapeutic target.

2377 Astrocyte Specific Expression of DJ-1 Attenuates Rotenone-Induced Neurotoxicity: A Gene Therapy Approach

B. R. De Miranda, E. Burton and J. Greenamyre. *Neurology, University of Pittsburgh, Pittsburgh, PA.*

Idiopathic Parkinson's disease (PD) etiology is commonly postulated as an interaction between genetic susceptibility factors and environmental exposures. One of the causative genes is DJ-1, a protein found in reactive astrocytes surrounding the sites of major pathological damage in PD, the substantia nigra (SN) and the striatum (ST). Several lines of evidence indicate that DJ-1 is protective against oxidative stress; however, it may also play a role in mitochondrial function, and inhibit aggregation of the PD-related protein α-synuclein. In this context, we hypothesized that astrocyte-specific overexpression of DJ-1 protein in the SN and ST would protect against nigostriatal dopamine cell loss produced by the mitochondrial toxin rotenone. To test this, adult male Lewis rats were injected with a lentiviral vector (MuLV pseudotype) with specific tropism for astrocytes, which permitted overexpression of human DJ-1 protein (hDJ-1) or GFP (control) in transduced astrocytes. Following unilateral stereotaxic injection of the MuLV vector into the SN and dorsolateral ST, rats received daily rotenone treatment (2.8 mg/kg) until their neurobehavioral endpoint was reached. Histological analyses indicated that hDJ-1 transgene expression was successfully targeted to astrocytes within the SN and ST, and was excluded from other cell types, including microglia and neurons. Rotenone toxicity resulted in dopamine neuron loss within the SN, assessed via stereology, and nerve terminal loss in the ST; however, this pathology was attenuated by overexpression of astrocytic hDJ-1. Neuroprotection was also extended to the mitochondria of dopamine neurons, where protein markers for Complex I were significantly preserved in animals expressing the hDJ-1 transgene ($p<0.01$). These results indicate that a targeted and cell-specific gene therapy approach overexpressing human DJ-1 may be a therapeutic strategy for combating gene-environment interactions in PD. Supported by ES020327 and T32 NS086749.

2378 Activation of the Nuclear Receptor Nur77 by a Novel Diindolylmethane Analog Suppresses Inflammatory Gene Expression in Primary Astrocytes

K. A. Popichak[1], S. L. Hammond[1], S. H. Safe[2] and R. B. Tjalkens[1]. [1]*Colorado State University, Fort Collins, CO and* [2]*Texas A&M, Houston, TX.*

Inflammatory activation of glial cells promotes loss of dopaminergic neurons in Parkinson's disease (PD). The transcription factor, Nuclear Factor -kappa B (NF-κB), regulates the expression of multiple neuroinflammatory genes associated in activated glial cells including inducible nitric oxide synthase (iNos), Tnfα and Il1β. These observations suggest that inhibition of NF--κB in glial cells could be a promising therapeutic target for the prevention of neuroinflammatory injury. Nuclear orphan receptors in the NR4A family, including NR4A1 (Nur77) and NR4A2 (Nurr1), can inhibit the inflammatory effects of NF-κB but there are no approved drugs that target these receptors. We postulated that a novel ligand of Nur77, 1,1-bis (3'-indolyl)-1-(p-methoxyphenyl) methane (C-DIM5), would suppress NF-κB-dependent inflammatory gene expression in astrocytes induced by 1-methyl-4- phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and the inflammatory cytokines IFN-γ and TNF-α. C-DIM5 increased expression of Nur77 mRNA and suppressed expression of neuroinflammatory genes. C-DIM5 inhibited the expression of multiple NF-κB-regulated inflammatory and apoptotic genes in qPCR array studies but did not prevent p65 translocation to the nucleus, suggesting a nuclear specific mechanism of inhibition. For the first time, we show that MPTP shuttles Nur77 from the nucleus of astrocytes to the cytoplasm, while C-DIM5 keeps Nur77 sequestered in the nucleus. These data demonstrate that C-DIM5 prevents the production of neurotoxic inflammatory mediators in glial cells through inhibition of NF-κB at a nuclear level, suggesting that this series could be a useful modality in preventing neuroinflammation.

2379 A Potential Role for Phosphotidyl-Inositol-3-Kinase (PI3K) in Regulating Manganese Transport through an Autophagy-Mediated Mechanism

M. R. Bryan[2], M. A. Uhouse[2], A. M. Tidball[2], T. J. Bichell[2], G. Tipps[2], E. Bradley[2], M. Aschner[1] and A. Bowman[2]. [1]*Einstein College, Bronx, NY and* [2]*Neurology, Vanderbilt University Medical Center, Nashville, TN*. Sponsor: A. Bowman.

Manganese (Mn), while toxic in excess, is an essential metal in almost all biological systems. Recently, we have shown that cellular models of Huntington's disease (HD) exhibit decreased Mn uptake that manifests as a Mn-selective deficit in ATM-dependent phosphorylation of p53. We sought to explore the relationship of other critical cell signaling proteins which have been implicated in HD etiology and Mn toxicity (PI3K, AKT, mTOR) at sub-toxic levels of Mn and whether these pathway contribute to neuronal Mn homeostasis. We first screened several small molecule inhibitors (SMIs) of PI3K and PIKKs (phosphotidylinsolitol three-kinase-related kinases) to determine if any of these kinases can function to modulate Mn homeostasis. Our data suggest that Mn uptake can be drastically reduced (>50%) in STHdhQ7/Q7 and STHdhQ111/Q111 cells via selective inhibition of PI3K only. Given these findings, we then examined the responsiveness of these pathways to sub-toxic concentrations of Mn. We observed that STHdhQ111/Q111 show diminished activation of these pathways in the presence of Mn. Interestingly, Mn-dependent activation of p53, AKT and S6 in HD STHdhQ111/Q111 cells can be restored to that seen in wild-type cells following exposure with Mn and 10uM KB-R7943, an inhibitor of NCX1/3. Considering that autophagy functions downstream of the PI3K/AKT/mTOR pathways, we investigated whether KB-R7943 acts through autophagy to increase Mn-uptake. Dual KB-R7943/Mn exposures increase expression of P62 and LC3-II/I expression, indicative of an increase in autophagy in both STHdhQ7/Q7 and STHdhQ111/Q111 cells and the striatum of the YAC128 mouse model. Consistent with our inhibition of Mn-uptake by PI3K inhibition, these findings could suggest that Mn uptake may be regulated via an autophagy-mediated route, controlled by upstream kinases including PI3K. Furthermore, mutant HTT's ability to inhibit autophagy could explain why both striatal cellular and mouse models exhibit a decrease in Mn-uptake. Together, these findings implicate a novel pathway in neuronal Mn homeostasis and suggests that restoration of these homeostatic pathways could potentially alleviate cell signaling defects common to most HD models. Supported by NIH ES016931 (ABB) and ES010563 (ABB and MA)

2380 Dopamine and L-DOPA Induced Post-Translational Modifications of Alpha-Synuclein

S. S. Lau, A. B. Cholanians and T. J. Monks. *Pharmacology and Toxicology, The University of Arizona, Tucson, AZ.*

Alpha-Synuclein (SYN) plays a central role in synucleinopathies and comprises the major component of aggregates present in Lewy Bodies, a major hallmark of Parkinson's disease (PD). Moreover, dopamine (DA) mediated SYN post-translational modifications (PTM) play an important role in the etiology of the disease, although the consequences of such PTMs remain the subject of debate. The DA precursor, L-DOPA, remains the drug of choice for the treatment of PD. We therefore compared the effects of L-DOPA and DA on SYN aggregation. Western blot analysis of L-DOPA and DA-modified SYN revealed that both compounds stimulated SYN aggregation, although the pattern of the PTMs differed. MALDI-TOF analysis of DA-modified SYN revealed an addition of 4.5 kDa to the SYN monomer, and up to 9 kDa to the dimer, suggesting poly-DA interactions with SYN, crosslinking of protein, and aggregation of SYN. Since there are no cysteine residues in SYN, modification by DA and/or L-DOPA primarily involves either oxidation of methionine or adduction of amino groups in lysine (K). We have previously shown that quinones preferentially form adducts with K-rich proteins, and in particular with proteins expressing KXK or XKKX motifs, such as SYN. Acetylating SYN-K residues, and peptides containing the KXK or XKKX motifs substantially ameliorated DA-induced SYN aggregation. DA and L-DOPA were conjugated with GSH and/or NAC, removing one of the reactive sites from the aromatic ring, which greatly limits the potential for post-adduction chemistry and reduces the complexity of the subsequent MS analysis. Mono-thiol conjugates of L-DOPA and DA oxidize and generate SYN adducts in a pH-dependent manner similar to unadducted DA, but fail to generate insoluble SYN aggregates. The findings reveal that scavenging of L-DOPA and DA quinones by cysteine and/or GSH has profound effects on their interaction with SYN, and on subsequent aggregation reactions. Thiol modulation of SYN PTMs and of SYN aggregation suggests potential interventions to alleviate SYN-dependent cytotoxicity.

2381 Preferential Extracellular Generation of the Active Parkinsonian Toxin MPP+ by Transporter-independent Export of the Intermediate MPDP+

S. Schildknecht, R. Pape and M. Leist. *University of Konstanz, 78457 Konstanz, Germany*. Sponsor: M. Leist.

The widely applied experimental protoxin 1-methyl-1,2,3,6-tetrahydro-pyridine (MPTP) causes Parkinsonian symptoms by killing a subpopulation of dopaminergic neurons in mice, primates and humans. Despite the wide use of MPTP, the mechanisms resulting in the preferential extracellular generation of the membrane-impermeable active toxin 1-methyl-4-phenyl-pyridinium (MPP+) has not satisfactorily been elucidated yet. We addressed the two following questions: 1) Why are MPTP converting astrocytes largely spared from MPP+ toxicity? 2) How does the membrane-impermeable MPP+ reach the extracellular space? In MPTP converting astrocytes, we detected MPP+ formation almost exclusively in the extracellular, but not in the intracellular space. The intermediate MPDP+ was identified as the primary product of the monoamine oxidase B (MAO-B) catalyzed conversion of MPTP. Under physiological conditions, MPDP+ partially exists in its uncharged conjugate base form 1.2-MPDP. We observed that the intermediate MPDP+ and/or 1.2-MPDP can passively diffuse across cell membranes and that MPP+ is preferentially formed in the extracellular space by autoxidation. These findings allow an explanation for the global MPTP conversion and formation of extracellular MPP+ in the brain even in areas lacking significant expression of transporters such as the Organic Cation Transporter 3 (OCT3) that was previously suggested to mediate MPP+ export from astrocytes. We noticed that the non-enzymatic autoxidation of MPDP+/1.2-MPDP into MPP+ was favored by conditions prevailing in the extracellular space such as a more alkaline pH or higher oxygen levels. This preferential extracellular autoxidation not only prevents the formation and accumulation of MPP+ within astrocytes, but is also a prerequisite for the well-established transporter-dependent neuronal uptake of MPP+ and the compound's selective toxicity in subpopulations of catecholaminergic neurons.

2382 Striatal Arginase Activity Deficit Is Ameliorated by Mn Exposure in HD Model Mice

K. G. Tipps[1], T. J. V. Bichell[1,2], E. M. Bradley[1] and A. B. Bowman[1,2]. [1]*Neurology, Vanderbilt University Medical Center, Nashville, TN and* [2]*Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN.*

Manganese (Mn) is an essential metal that is both critical for neuronal function and toxic in excess. Altered Mn homeostasis has been implicated in the pathophysiology of some neurological diseases, including Huntington's disease (HD). Expansion of the polyglutamine tract in Huntingtin (HTT) leads to selective neurodegeneration of the striatum in HD. Expression of mutant HTT is also associated with a manganese accumulation deficit in the striatum of YAC128Q mice, an HD model. Thus, impaired neuronal Mn handling may contribute to HD pathophysiology. Arginase is a Mn-dependent enzyme that plays a central role in the urea cycle. We have optimized a sensitive arginase activity assay that measures arginine-dependent urea production in order to detect changes in arginase activity in the striatum of prodromal (3 month) YAC128Q mice under basal conditions, or with elevated *in vivo* Mn levels via a one-week Mn exposure paradigm in which mice are injected subcutaneously with 13.88 mg/kg Mn. We detected a significant reduction in basal arginase activity in the striatum of HD mice versus wild type. Mn exposure increased arginase activity by five-fold in WT mice and by thirty-two-fold in HD mice, rescuing the baseline deficit. Exogenous supplementation of Mn to 2.5 mM in the assay also ameliorated the genotype difference, increasing activity by six-fold in WT mice and by forty-fold in HD mice. In conclusion, we have optimized an arginase activity assay to allow detection of changes in striatal arginase activity in an HD mouse model with *in vivo* and *in vitro* Mn exposure, both of which normalize activity between the genotypes, thereby rescuing a baseline activity deficit in the HD mice. These data support the hypothesis that impaired striatal Mn handling may contribute to HD pathophysiology. Supported by RO1 ES016931, RO1 ES010563, and T32 ES007028

2383 The Benefits of Swimming Exercise on the Aging Brain and its Underlying Mechanism

F.-H. Yang[3], Y.-H. Yang[3], Y.-C. Chuang[3], S.-Y. Tong[3], J. D. Chen[3], Y.-C. Huang[1], M.-W. Chao[3] and C.-Y. Tseng[3]. *[1]Taoyuan General Hospital, Taoyuan, Taiwan; [2]Department of Biomedical Engineering, Chung Yuan Christian University, Taoyuan, Taiwan and [3]Department of Bioscience Technology, Chung Yuan Christian University, Taoyuan, Taiwan.*

Oxidative stress contributes to the aging process is a well-known issue. Brain is highly sensitive to oxidative stress according to its high metabolic rate and low antioxidant ability. Research has shown that age-induced oxidative stress causes the reduction in hippocampal neurogenesis and further results in neurodegeneration, following by abnormal brain function. Exercise has been recommended to improve the life quality by maintaining better health condition. Evidence also shows that exercise-trained rats have greater bone mass, density, strength, and dynamics; Swimming exercise is displayed a hepatoprotective effects; Physical exercise improves the protein function by down-regulating aging induced protein turnover rate, and enhances spatial memory, cognitive functions and plasticity. Accordingly, exercise may act as a ROS scavenger that reduces the aging caused oxidative stress. To address above hypothesis, D-galactose (100 mg/kg/day) induced aging model is adopted together with natural aging animals. We have found that aging and exercise have no effects in locomotor activity and anxiety tested by open field test. Aging causes the impairment in spatial hippocampal-dependent memory in spatial object recognition test. Morris water maze results show that consecutive exercise significantly improves the spatial hippocampal-dependent memory in D-gal induced aging rats. Moreover, there is a significantly reduction in antioxidant enzyme expression both in natural and D-gal aged rats, and exercise is able to inhibit these decreases. This study addresses that the consecutive days of exercise rather than post exercise during aging processes leads to better in the maintenance of cognitive function.

2384 Increased Respiration and Acidosis in a FUS Overexpression Model of Amyotrophic Lateral Sclerosis

K. L. Jackson[2], H. A. Dhaibar[2], R. D. Dayton[2], S. G. Cananzi[2], W. G. Mayhan[2], A. E. Glasscock[2] and R. L. Klein[2]. *[1]Dept. of Cellular Biology and Anatomy, LSU Health Sciences Center, Shreveport, LA and [2]Dept. of Pharmacology, Toxicology, and Neuroscience, LSU Health Sciences Center, Shreveport, LA.* Sponsor: K. McMartin.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that causes a progressive paralysis due to the death of upper and lower motor neurons. The majority of patients with ALS die from respiratory failure and respiratory complications. Despite the vital importance of the diaphragm and consequently respiratory function in ALS, respiration in ALS rodent models is rarely studied. Mutations in fused in sarcoma (FUS), a DNA- and RNA-binding protein, can cause familial ALS, and the overexpression of FUS is neurotoxic. We administered adeno-associated virus serotype 9 (AAV9) encoding FUS to adult female rats and analyzed weight, motor function (escape reflex, rotarod, locomotor activity, forelimb grip strength), and respiratory parameters (whole body plethysmography) over a period of eight weeks. These rats developed progressive motor impairments with sustained hyperventilation and acidosis during the end stage of the disease. These data support that respiratory changes are a powerful and highly relevant readout for preclinical therapeutic testing.

2385 Environmental Exposure to Lead (Pb) and Tau Pathology *In Vitro*

A. Eid[1], N. Zawia[1] and S. Waseem[2]. *[1]Interdisciplinary Neurosciences, University of Rhode Island, Kingston, RI and [2]Pharmacology and Toxicology, University of Hail, Hail, Saudi Arabia.*

Alzheimer's Disease (AD) is characterized by the abnormal excessive accumulation of two pathological deposits in the brain, Aβ which is preceded by the amyloid precursor protein (APP) and the other, neurofibrillary tangles (NFT) composed of hyperphosphorylated microtubule associated protein tau (MAPT). Late onset AD (LOAD) makes up more than 95% of individuals diagnosed with the disease. These individuals are typically aged 65 years or older, and have no specific genetic mutation that can be linked to the disease. We believe that a major contributor to the development of LOAD could be an environmental component. Previous studies by us have shown upregulation of proteins involved in Aβ pathology in SHSY5Y cells exposed to lead (Pb) (Bihaqi 2012). Specifically, we have shown upregulation of APP, BACE1, and SP1, 6 days after exposure was ceased (Bihaqi 2012). The objective of the current study is to examine the effects of environmental exposure on tau pathology in human neuroblastoma cells. SHSY5Y cells were differentiated for 6 days using all trans-retinioic acid. They were exposed to a series of Pb concentration (0, 5, 50, 100 μM) for 48 hours. Samples were collected 6 days after exposure in order to model a latent scenario. Results from these studies e show that exposure to Pb at 50 and 100μM doses increases total Tau levels. Site specific phosphorylation at Thr-181 and Ser-235 also displayed a significant increase at 100 μM Pb concentration. Similar increases were also found for Thr-212 and Ser-396 at both the 50 and 100 μM Pb concentration. We have also begun to scrutinize the effects of other toxicants, such as lipopolysaccharide (LPS, 2μg/mL) and manganese (Mn, 10μM), using the same exposure paradigm. Our initial and preliminary results indicate that LPS and Mn increase total tau levels relative to control. These findings implicate environmental exposures with proteins that have been heavily involved in AD. Suggesting a role for these toxicants in the etiology of the late-onset form of this neurodegenerative disorder.

2386 Accumulation of Beta-Amyloid 1-42 in Hippocampus of Rats Chronically Exposed to Low Ozone Doses: a Model of Alzheimer's Disease

S. Rivas-Arancibia and E. Rodriguez-Martinez. *Fisiologia, Universidad Nacional Autonoma de México, Mexico DF, Mexico.* Sponsor: S. Rivas-Arancibia.

Environmental pollution presents a major health problem in densely populated cities, the ozone air pollution is a serious health problem because it causes a state of oxidative stress. Which it is present in the chronic degenerative diseases, on the other hand, exposure to an oxidizing environment is correlated with a large increase in the number of consultations and exacerbations of chronic degenerative diseases in hospitals. The objective of this work was to study the formation and accumulation of beta-amyloid 1-42 (A-β 1-42), which is associated with oxidative stress and loss of regulation of the inflammatory response, as well as changes in processing enzymes of the amyloid precursor protein (APP) caused by exposure to low doses of ozone. For this purpose we used 36 rats of the Wistar strain, housed in individual box acrylic, with free access to food and water and air ozone free, the animals were randomly divided into 3 groups, and each group received one of the following treatments: 1) control group, exposed to an air flow, free of ozone; 2) Group exposed to ozone for 60 days; 3) Group exposed to ozone for 90 days. Exposure to ozone was performed from Monday to Friday for 4 hours per day to 0.25 ppm. Two hours after the treatment was finished, the animals were deeply anesthetized with sodium pentobarbital (50 mg / K). Six animals from each group were processed for immunohistochemical techniques and the remaining 6 for western blot. The following antibodies were used. AB 1-42 and AB-1-40, APP, ADAM 9, ADAM 10, presenilin 1, presenilin 2, interleukin 1, interleukin 17, interleukin 10: The results indicate a significant accumulation and increased of A-β 1-42 and a significant rise in the presenilin 1 and 2, as well as IL 1 IL17 increase ($p < 0.05$). Besides, it presents a decrease in both the A-β 1-40, as well as IL10, ADAM 9 and 10 ($p < 0.05$)In addition, there was a decrease in both the A-β 1-40, as well as IL10, ADAM 9 and 10 ($p < 0.05$). With the obtained results, we can conclude that chronic exposure to low doses of ozone causes oxidative stress and loss of regulation of the inflammatory response which induces the amyloidogenic pathway activation with the formation of A-β 1-42 in healthy animals without any other added factor, these changes are similar to what is observed in Alzheimer's disease. This project was financed to S R-A by DGAPA N° IN221114 and CONACYT N° 219703

2387 siRNA Based Silencing of Mammalian Target of Rapamycin in LUHMES Cell Model of Tauopathy

M. Salama[1], M. El-Husseiny[1], R. El-Ashry[1], N. Badawy[1] and W. Mohamed[4]. *[1]Mansoura-Manchester Medical Program, Mansoura University, Mansoura, Egypt; [2]MERC, Mansoura University, Mansoura, Egypt; [3]Pathology, Mansoura University, Mansoura, Egypt and [4]Pharmacology, Menoufia University, Menoufia, Egypt; [5]Toxicology, Mansoura University, Mansoura, Egypt.*

Studying neurodegenerative disorders is becoming increasingly important. One group of such neurodegenerative diseases collectively referred to as tauopathies. In tauopathies, the soluble tau protein detaches from microtubules and forms abnormal aggregates of hyperphosphorylated, and ubiquinated tau. Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that regulates processes including mRNA translation, proliferation, and survival. As a central element signaling cell growth and enhancing protein translation, (mTOR), when inhibited, induces

autophagy. The phosphatidylinositiol 3-kinase (PI3K), AKT, mammalian target of rapamycin signaling pathway (PI3K/AKT/mTOR) is frequently dysregulated in disorders of cell growth and survival, and aging diseases especially neurodegenration. In the present study, we explored the consequences of mTOR deletion in tauopathy model in human postmitotic dopaminergic neurones (LUHMES). Tauopathy was induced in the cell culture by adding the mitochondrial complex I inhibitor (Piericidine A1). siRNA sequences were validated to efficiently knock down mTOR in the cell culture. Then,siRNA transfection of cell culture was done by means of Accell-siRNA.we studied the biological consequences of mTOR knockdown in these cells regarding mTOR and other autophagy associated proteins, mitochondrial energy metabolism as well as phosphorylation, somatodendritic re-distribution and aggregation of the tau protein(cells were immunostained with the mouse monoclonal antibody AD2 raised against pS396/pS404 phosphorylated tau; the percentage of neurons in which AD2-immunoreactivity was redistributed from the axons to the cell soma was quantified in 5 visual fields). Our Results showed that following efficient blocking of mTOR, there was proved protection as regards the number of abnormal aggregates of phosphorylated tau, and the percentage of neurons in which tau was redistributed from axons to cell soma. Moreover, there was efficient decrease in cell death following siRNA transfection compared to cells treated with piericidine A1 only. This work was supported by a grant from Parkinson's and Movement Disorders Foundation (PMDF).

2388 A Comparative Study on the Effects of Maneb, Copper, and Their Combination on Rat Hippocampal Astrocytes and Mouse Neuroblastoma Cells

H. A. Ebid and L. D. Trombetta. *PHS, St. John's University, Queens, NY.*

Maneb, Mn-ethylene bisdithiocarbamate fungicide, has been linked with the development of neurodegenerative diseases. Copper, a redox transition metal, is vital to many cellular processes. Increased copper content however has been linked with neurodegenerative changes. Neurotoxicity of maneb and of high levels of copper has been attributed to their ability to induce oxidative stress. Previous studies have shown that ethylene bisdithiocarbamates such as maneb can chelate metals such as copper forming a complex that is highly lipophilic and can easily cross cell membranes. In this study we hypothesized that such a complex might elevate the intracellular levels of copper and therefore increase the neurotoxicity. The purpose of this study was to investigate whether the co-exposure of rat astrocytes and mouse neuroblastoma to maneb plus copper can be more neurotoxic than exposure to maneb or copper alone. Cell viability test was conducted on both astrocytes and neuroblastoma cells using Trypan blue exclusion assay. Both cell lines were treated at sub-confluency and incubated for 24 hours. Astrocytes were exposed to maneb (0, 2.5, 5, 10, 15, 20, 25 μM), Cucl2 (0, 500, 1000, 1500, 2000 μM) or combination of maneb (14 μM) plus Cucl2 (100, 200, 300, 400, 500 μM). Neuroblastoma cells were exposed to maneb (0, 2.5, 5, 10, 15, 20, 25 μM), Cucl2 (0, 200, 400, 600, 800, 1000 μM) or combination of maneb (9 μM) plus Cucl2 (1, 3, 5, 7, 8, 10 μM). From the cell viability results, we estimated LC50 of maneb to be 14 μM for astrocytes and 16 μM for neuroblastoma, LC50 of Cucl2 to be 1001.7 μM for astrocytes and 601.6 μM for neuroblastoma, and LC50 of the combination to be 14 μM of maneb + 166.1 μM of Cucl2 for astrocytes and 9 μM of maneb + 2.5 μM of Cucl2 for neuroblastoma. From our results we concluded that maneb increased the neurotoxicity of copper on both cell lines and that astrocytes were more resistant against the neurotoxic effects of the combination compared to neuroblastoma.

2389 Abstract Withdrawn by Author.

2390 Acute Low-Level Chlorpyrifos Exposure Alters Capsaicin Response in Dorsal Root Ganglion Neurons

H. A. Enright[2], S. H. Felix[2], E. Mukerjee[2], N. O. Fischer[2], D. Soscia[2], A. Kopacz[2], K. Kulp[2] and E. K. Wheeler[2]. [1]*Biosciences and Biotechnology Division, Lawrence Livermore National Lab, Livermore, CA and* [2]*Engineering, Lawrence Livermore National Lab, Livermore, CA.*

Chlorpyrifos (CPF) is a well-known organophosphorus insecticide that is widely used in both agriculture and industry. CPF has been documented to cause toxicity in many organs and systems, including the peripheral and central nervous systems. Similar to other organophosphates, CPF inhibits acetylcholinesterase (AChE) in the nervous system, which leads to elevated levels of acetylcholine resulting in cholinergic toxicity. CPF exposure may be associated with organophosphate induced delayed polyneuropathy (OPIDP), a delayed neurotoxic effect that typically occurs 1–5 weeks post exposure. While some cases have been reported in humans, there is limited data on the mechanism for pain signaling, especially after low level exposure. In this study, we investigated whether CPF exposure affects pain signaling in rat dorsal root ganglion neurons using our multi-electrode array (MEA) platform. Long-term, non-invasive assessment of neuronal health and function was investigated after acute CPF exposure. Compared to controls, CPF treated cultures showed a reduction in electrophysiological response to capsaicin stimulation. The number of spikes provoked by capsaicin was reduced although no significant differences were observed for median interspike interval or spontaneous activity between groups. Assessment of cell viability and capsaicin receptor distribution was also determined. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABS-677902

2391 Acute Exposure to Chlorpyrifos Causes Oxidative Stress in a Striatal Cell Model of Huntington's Disease

G. Kwakye and G. Dominah. *Neuroscience, Oberlin College, Oberlin, OH.*

In spite of the genetic cause of Huntington's disease (HD), emerging evidence suggests that potential exposure to environmental factors may contribute to the variability in age of onset, progression and severity of the disorder. However, the identity of the environmental risk factor is currently unknown. Recognizing some of the shared similarities in the pathophysiological mechanisms between HD and pesticide neurotoxicity such as oxidative stress and mitochondrial dysfunction, we hypothesized that the commonly used agrochemical chlorpyrifos (CPF) would exhibit a disease-toxicant interaction and reveal the influence of CPF in HD neuropathology. Following a 48-hr exposure to the metabolites CPO and TCP, we report no significant dose and genotypic differences in cell survival. However, mutant STHdhQ111/Q111 cells show increased susceptibility to CPF neurotoxicity compared to wild-type STHdhQ7/Q7 after 48-hr exposure. In addition, mutant STHdhQ11/Q111 cells exhibit significant increased production of reactive oxygen species and diminished glutathione levels in a dose-dependent manner compared to wild-type STHdhQ7/Q7 as early as 20 minutes exposure. We examined the possibility of an antioxidant, N-acetylcysteine (NAC), to ameliorate the genotype-dependent difference in cell survival. Pre- and simultaneous, but not post treatments of striatal cells with 100 μM NAC was sufficient to decrease CPF-induced toxicity and abolish the genotypic difference in the striatal HD cell survival. Furthermore, we report increased heme-oxygenase 1 (HO-1), a biomarker for oxidative stress, in both genotypes after 48-hr exposure. These results suggest that expression of mutant HD protein may exacerbate CPF neurotoxicity via oxidative stress to cause striatal neuron loss.

2392 Acute Exposure to Chlorpyrifos Causes Mitochondrial Dysfunction in a Striatal Cell Model of Huntington's Disease

R. A. McMinimy, S. Kallon, G. Dominah and G. Kwakye. *Neuroscience, Oberlin College, Oberlin, OH.*

An emerging perspective on the pathogenesis of neurodegenerative diseases such as Huntington's disease (HD) seeks to integrate knowledge of genetic susceptibility factors and environmental agents to better understand how they may interact to modify neuropathology. Despite the genetic etiology of HD, emerging evidence suggests that unknown environmental factors may modify the age of onset, disease progression, and symptomology of HD. Importantly, pesticide-induced neurotoxicity shares similar pathophysiological mechanisms with the genetic mutation that causes HD. Exposure to chlorpyrifos (CPF), an organophosphate pesticide known to inhibit acetylcholinesterase (AChE) activity has been reported to impair mitochondrial function independent of AChE inhibition. Here, we seek to better understand how exposure to CPF exposure coupled with the HD mutation may synergistically amplify the mitochondrial dysregulation characteristics of HD in an established striatal cell model of HD. We report that mutant striatal cells (STHdhQ111/Q111) are more susceptible to acute CPF neurotoxicity compared to wild-type striatal cells (STHdhQ7/Q7) using metabolic capability assays including colorimetric MTT and CellTiter-Blue, both of which measure mitochondrial respiratory function in live cells. In addition, JC-1 experiments revealed that mutant striatal cells exhibit greater mitochondrial membrane depolarization with parallel reductions in ATP production, revealed through the luciferin-lucferase assay, compared to

wild-type cells after 48 hours CPF exposure. Ongoing work is to decipher the cellular underpinnings of the disease-toxicant interaction in mitochondrial dynamics and trafficking. Our overarching research goal is to gain a better understanding of how exposure to CPF may potentially modify HD symptoms and promote earlier development of the disease.

2393 Acute and Long-Term Exposure to Chlorpyrifos Induces Cell Death of Basal Forebrain Cholinergic Neurons Through AChE Variants Alteration

P. V. Moyano Cires[2], M. T. Frejo[2], M. J. Anadon[2], J. M. Garcia[2], M. J. Diaz[2], G. Gomez[2] and J. Del Pino[2]. *[1]Toxicology and Legal Medicine, Complutense University of Madrid, Madrid, Spain and [2]Toxicology and Pharmacology, Complutense University of Madrid, Madrid, Spain.*

Chlorpyrifos (CPF) is one of the most widely used organophosphates insecticides that has been reported to induce cognitive disorders both after acute and repeated administration similar to those induced in Alzheimer's disease (AD). However, the mechanisms through which it induces these effects are unknown. On the other hand, the cholinergic system, mainly basal forebrain cholinergic neurons, is involved in learning and memory regulation, and an alteration of cholinergic transmission or/and cholinergic cell loss could induce these effects. In this regard, it has been reported that CPF can affect cholinergic transmission, and alter AChE variants, which have been shown to be related with basal forebrain cholinergic neuronal loss. According to these data, we hypothesized that CPF could induce basal forebrain cholinergic neuronal loss through cholinergic transmission and AChE variants alteration. To prove this hypothesis, we evaluated in septal SN56 basal forebrain cholinergic neurons, the CPF toxic effects after 24 h and 14 days exposure on neuronal viability and the cholinergic mechanisms related to it. This study shows that CPF impaired cholinergic transmission, induced AChE inhibition and, only after long-term exposure, increased CHT expression, which suggests that acetylcholine levels alteration could be mediated by these actions. Moreover, CPF induces, after acute and long-term exposure, cell death in cholinergic neurons in the basal forebrain and this effect is independent of AChE inhibition and acetylcholine alteration, but was mediated partially by AChE variants alteration. Our present results provide a new understanding of the mechanisms contributing to the harmful effects of CPF on neuronal function and viability, and the possible relevance of CPF in the pathogenesis of neurodegenerative diseases.

2394 Transcriptional Alterations of Cholinergic and Dopaminergic Genes in Mice Neonatally Exposed to the Combination of the Pesticides Carbaryl and Chlorpyrifos

H. Viberg, S. Hallgren, P. Hamberg and P. Eriksson. *Environmental Toxicology, Uppsala University, Uppsala, Sweden.*

Pesticides are detected in the environment, and humans, including placental cord blood, mothers' milk and blood in newborns and toddlers, meaning they can interfere with sensitive developmental periods of CNS. Organophosphates (OPs) and carbamates have the same mechanisms of action and we have recently seen that the carbamate carbaryl (CARB) and the OP chlorpyrifos (CPF) can affect the postnatal brain development, resulting in altered adult behavior and cognitive function. The cholinergic system is important for behavior, learning and memory and also complexly interacting with dopaminergic neurons, and both carbamates and OPs affect central cholinergic and dopaminergic function. On postnatal day 10, male mice were exposed to a single oral dose of 20 mg CARB/kg, 10 mg CPF/kg or the combination of these compounds. 24 h or 2 months post treatment, brain tissues were analyzed for gene expression levels in the cholinergic and dopaminergic system. The expression of AchE was increased in neonatal hippocampus after CPF and combination exposure and mAChR-5 was decreased in neonatal striatum after combination exposure. In adult mice CARB and CPF increased gene expression of nAChR-β2 and mAChR-5, while all exposure groups showed decreased transcript levels of AChE, nAChR-α4, nAChR-α7, nAChR-β2, and mAChR-5 in striatum. Dopaminergic genes (DAT, DRD1, DRD2, TH) were affected in neonatal cortex and striatum. In adult mice, both cortex and striatum showed decreases in several dopaminergic genes (DAT, DRD1, DRD2, DRD5, TH), after neonatal exposure to CARB, CPF or the combination The results indicate that both CARB and CPF can affect the developing cholinergic and dopaminergic systems by altering gene transcription, which can be the mechanisms behind the adult behavioral disturbances. Furthermore, combination exposure of these two AChE inhibitors seem to have a more severe effects on gene expression. The present results call for increased awareness of possible neurotoxic interaction between different pesticides.

2395 The Antioxidant Trolox Prevents Learning and Memory Deficits in a Rat Model of Subchronic Chlorpyrifos (CPF) Exposure

D. A. Bruun, S. N. Levoe, K. Streifel, B. Flannery and P. J. Lein. *Molecular Biosciences, University of California Davis, Davis, CA.*

CPF is an organophosphorus pesticide that is widely used in agricultural and commercial applications. Occupational exposures to CPF have been associated with deficits in cognitive and psychomotor performance; however, the mechanism(s) by which such exposures cause neurotoxicity remain speculative. In this study, we tested the hypothesis that oxidative stress contributes to CPF-induced learning and memory deficits. Based on exposure data collected from 255 Egyptian agricultural workers responsible for applying CPF to cotton fields, adult male Long Evans rats were exposed to 10 mg/kg/d CPF or vehicle for 21 d. When assessed immediately after the 21 d exposure period, CPF-exposed animals exhibited significant deficits in contextual fear conditioning, which is a test of learning and memory. Increased levels of PGE2 in the serum and brain as well as increased tyrosine nitration in the hippocampus and cortex were observed at earlier time points preceding the onset of behavioral deficits but there were no treatment-related differences at the time of behavioral testing. Treatment with Trolox (1 mg/kg/d ip) beginning 5 d prior to CPF exposure and continuing throughout the 21 d CPF exposure blocked the increase in serum PGE2 levels and prevented deficits in contextual fear conditioning. Treatment with Trolox did not prevent the effects of CPF on AChE activity in the serum or brain. Results from this study indicate that chronic CPF exposure causes a transient increase in oxidative stress that is causally linked to learning and memory deficits. These findings suggest biomarkers of oxidative stress as potential biomarkers of effect for chronic OP neurotoxicity and identify a potential approach for protecting workers occupational exposed to OPs. Supported by the NIEHS (ES016308), the NINDS CounterACT program (NS079202) and a postdoctoral fellowship to KS (NIH ES024676).

2396 Stat-1 Is a Critical Regulator of Chlorpyrifos-Induced Cell Death in Dopaminergic Neuronal Cells

N. Singh, P. Phan, V. Lawana, H. Jin, V. Anantharam, A. Kanthasamy and A. Kanthasamy. *Biomedical Sciences, Iowa State University, ames, IA.*

Numerous epidemiological studies link chronic exposure to pesticides to the progression of neurological disorders including Parkinson's disease (PD). Chlorpyrifos (CPF), an AchE inhibitor, has been implicated in dopaminergic neurotoxicity; however, the precise mechanism by which it induces neuronal cell death remains unclear. In the present study we sought to investigate the mechanistic basis of CPF-induced cytotoxicity in N27 cell, an immortalized dopaminergic cell line and primary microglial cells. Exposure of dopaminergic neuronal cells to increasing concentrations (10-500μM) of CPF-induced a dose dependent reduction in cell viability which was accompanied by enhanced ROS generation, caspase-3 activation, proteolytic cleavage of proapoptotic PKCδ, DNA damage, & concomitant reduction in GSH levels, mitochondrial membrane potential (MMP) and ATP levels. Mechanistic studies revealed that CPF(50μM) also induced STAT1 (Signal transducers and activators of transcription) activation as evidenced by a time dependent increase in the phosphorylation of STAT1 on tyrosine residue 701 & Serine 727 and subsequent nuclear translocation of STAT1. Given the pivotal role of JAK-STAT signaling events in various biological processes including cell death, we examined the role of STAT-1 in CPF-induced apoptotic cell death. Intriguingly, siRNA-mediated down regulation of STAT1 levels afforded significant protection against CPF-induced cell death in dopaminergic neuronal cells by significantly attenuating drug-induced upregulation of NOX-1 levels & subsequent ROS generation and associated caspase 3 activation, PKCδ activation, and PARP cleavage. Also, gene silencing of STAT1 decreased the impairment in mitochondrial function, attenuated GSH depletion, NOX upregulation and PARP cleavage. Collectively, the present study identifies STAT1 as a critical regulator of CPF-induced dopaminergic cell death that may be mediated, atleast in part, via PKC delta activation, impaired cellular redox machinery, and PARP cleavage. (Supported by NIEHS1058667).

2397 The Effects of Occupational Chlorpyrifos Exposure in Alzheimer's Disease Development

J. R. Voorhees[1,4], M. T. Remy[4], L. McDaniel[4] and A. A. Pieper[1,2,4]. [1]*Department of VA Center for the Prevention and Treatment of Visual Loss, Iowa City, IA;* [2]*Neurology, Iowa City, IA;* [3]*Department of Psychiatry, University of Iowa, Iowa City, IA and* [4]*Interdisciplinary Human Toxicology, University of Iowa, Iowa City, IA.* Sponsor: L. Robertson.

Organophosphates (OPs) are used extensively worldwide as powerful insecticides in agriculture and vector-borne disease control. At high exposure levels, OPs potently inhibit acetylcholinesterase, making them acutely toxic. However, the effects of OPs at lower, prolonged exposures, such as occupational exposures, are poorly understood. Epidemiology studies suggest that repeated, low level OP exposure is harmful to human health and that the central nervous system is vulnerable to these exposures. Such studies have identified associations between occupational OP exposures and neurologic disorders, including neurodegeneration, psychiatric illness, and deficits in cognition, such as memory, visual attention, and motor coordination. Among the neurodegenerative diseases associated with occupational OP exposure is Alzheimer's disease (AD), the most prevalent form of neurodegeneration. Here, we aim to clarify the relationship between occupational OP exposure and AD. We hypothesize that levels of the OP chlorpyrifos (CPF) modeling occupational exposure will perpetuate the severity and onset of AD-associated behaviors and pathologies. To test our hypothesis, a transgenic AD rat model, TgF344-AD, was exposed to a model of occupational CPF exposure and modifications in AD pathophysiological hallmarks and associated behaviors were examined. Results indicate that CPF exposure induces anxiety-like behaviors indiscriminately, but induces memory deficits in AD males only. Interestingly, sex and age related differences are evident in the analyzed behaviors. Neurite outgrowth and mitochondrial deficiencies are potential mechanisms underlying these behavior effects. Our hope is that results from this study will add to the literature used to determine regulations governing OPs, while also contributing to our understanding of the interaction between environmental toxicants and neurodegeneration.

2398 Molecular Dynamics Simulations of Patatin17 and the Patatin Domain of Neuropathy Target Esterase in Complex with Organophosphorus Compounds

S. Wijeyesakere[2] and R. J. Richardson[2]. [1]*Environmental Health Sciences, University of Michigan, Ann Arbor, MI and* [2]*Microbiology and Immunology, University of Michigan, Ann Arbor, MI.*

Neuropathy target esterase (NTE) is a member of a family termed the patatin-like phospholipase domain containing (PNPLA) proteins. NTE inhibition by organophosphorus (OP) compounds that undergo post-inhibitory aging triggers a sensorimotor distal axonopathy called OP compound-induced delayed neuropathy (OPIDN). In contrast, NTE inhibition by non-aging compounds does not produce OPIDN but protects against OPIDN from subsequent dosing of ageable OP compounds. OP inhibition of NTE involves organphosphorylation of the active site serine, whereas aging involves post-inhibitory loss of a phosphoryl ligand resulting in a negatively charged organophosphoryl moiety covalently linked to the active site serine. Lacking experimental structural data on full-length NTE, analysis of the crystal structure of patatin-17 (pat17) in complex with OP compounds along with simulated inhibition (SI) and aging (SA) of the patatin domain of NTE (PNTE) were used to test the hypothesis that inhibition and/or aging produce conformational changes. SI involved building a covalent diisopropylfluorophosphate (DFP) adduct on PNTE while SA involved removal of an isopropyl side-chain from the DFP adduct. All structures were analyzed via molecular dynamics (MD) simulations in YASARA. Similar to previous findings from the crystal structures of OP-inhibited and aged pat17, SI and SA of DFP adducts of PNTE did not yield measurable conformational changes in the protein relative to the native state (RMSD < 1 Å). However, analysis of the atomic motions in OP-bound pat17 and PNTE indicated that the inhibited forms of both enzymes were significantly less stable than their native or OP-aged counterparts. These insights into the relative effects of inhibition and aging on protein stability could not have been derived from static structures alone and indicate the potential value of molecular dynamics simulations in understanding effects of chemical modifications of proteins.

2399 Effects of Organophosphates Compounds on Neurite Outgrowth and on Cytoskeletal Components in Differntiated Human SH-SY5Y Neuroblastoma Cells

L. S. Fernandes[2], G. L. Emerick[1], A. C. Santos[2] and N. A. G. Santos[2]. [1]*Instituto de Ciências de Saúde, Universidade Federal do Mato Grosso – UFMT, Sinop-MT, Brazil and* [2]*Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo - USP, Ribeirão Preto - SP, Brazil.* Sponsor: G. Emerick.

The organophosphates(OP)compounds are one of class of pesticides more used worldwide. These compounds can cause a delayed neuropathy,known as organophosphorus-induced delayed neuropathy (OPIDN). To generate OPIDN it's necessary the inhibition and aging of at least 70% of the neuropathy target esterase (NTE). Other key early events leading to the expression of clinical OPIDN, include decrease of neurite outgrowth and disruption of the axonal cytoskeleton. The mature axons contain a high density of Neurofilaments(NFs), as the NF-200, which is a neuronal differentiation marker, and express also high levels of the growth-associated protein 43 (GAP43), which is expressed during axonal outgrowth. The aim of this study was to evaluate the effect of the OPs trichlorfon, mipafox (used as inducer of OPIDN) and paraoxon (used as non-inducer) in expression of GAP-43 and NF-200, and effect in neurite outgrowth in differentiated SH-SY5Y cells. The cell differentiation was induced by addiction of 10μM of RA for 5 days, and the differentiated cells were exposed for 72 hours to OPs at the concentrations that caused at least 70% of inhibition and aging of NTE and viewed using an inverted phase-contrast microscope. After obtaining of the images,cells were harvested from the wells and the cell lysate was made for analysis by western blot. For immunocytochemical analysis, the cells were incubated with anti-NF 200 and fluorescent intensity was examined under a fluorescence microscope. The results showed that mipafox and trichlorfon caused decreased of the average neurites length, in the percentage of cells with neurites and in the expression of GAP43 and NF-200 when compared with control group (only retinoic acid). The paraoxon also caused decreased in endpoints evaluated, however, it was a decreased lower when compared with the mipafox and trichlorfon. In conclusion, the three OPs caused decreased in the endpoints evaluated in this study, however, the paraoxon caused a decreased lower when compared with mipafox and triclorfon.

2400 Evaluation of the Structurally Similar Organophosphate Flame Retardants, Plasticizers, and Pesticides for Neurotoxic Potential

S. M. Marco[3], A. Tiethof[3], J. Richardson[1] and B. Buckley[3]. [1]*Dept of Pharmacy Practice, Northeast Ohio Medical University, Rootstown, OH;* [2]*Environmental and Occupational Health Sciences Institute, Rutgers University, Piscataway, NJ and* [3]*Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.*

Due to the phase-out of polybrominated diphenyl ether flame retardants, the use of alternatives such as organophosphate flame retardants (OPFRs) has increased. OPFRs are structurally similar to other organophosphate esters (OPEs) including organophosphate pesticides (OPs) and organophosphate plasticizers. The OPs are neurotoxic suggesting that based on their structural similarities, OPFRs and plasticizers may be neurotoxic as well. An indicator of OP induced neurotoxicity is the inhibition of critical enzymes such as acetylcholinesterase (AChE) and carboxylesterase. The purpose of our study was to screen several OPs, OPFRs, plasticizers, and their metabolites for potential AChE inhibition. Mouse brain homogenates were incubated with tris (2-chloroethyl) phosphate (TCEP; an OPFR), tributyl phosphate (TBP; a plasticizer), dibutyl phosphate (DBP; a metabolite of TBP), dichlorvos (DDVP; an OP), and chlorpyrifos oxon (CPO; an OP metabolite) for 30 minutes at 37°C. AChE activity was measured indirectly using a modified Ellman method, which quantified the concentration of 5-thio-2-nitrobenzoic acid ion formed by the reaction of 5,5'-dithiobis-2-nitrobenzoic acid and thiocholine iodide, a product of acetylthiocholine iodide hydrolysis by AChE. Control experiments using the known neurotoxicant CPO resulted in AChE inhibition values consistent with the literature. AChE inhibition values for 10, 5, and 1nM CPO were 75%, 57%, and 20%, respectively. 1mM TCEP, 1mM TBP, and 1mM DBP inhibited AChE by 51%, 4%, and 17%, respectively. 1mM and 200nM DDVP induced 95% and 43% AChE inhibition, respectively. All of the OPE classes tested inhibited some AChE activity, but the data show a clear stratification of the chemicals' inhibitory potential based on their functional groups.

2401 Occupational Pesticide Exposure and Parental Report of Attention Deficit Hyperactivity Disorder in Adolescent Pesticide Applicators in Egypt

D. S. Rohlman[1], A. Ismail[1], G. Abdel Rasoul[5], M. R. Bonner[3], O. Hendy[5], L. Ortega[1], K. Wang[1] and J. R. Olson[3]. *[1]Biostatistics, University of Iowa, Iowa City, IA; [2]Department of Epidemiology and Environmental Health, State University of New York, Buffalo, NY; [3]Department of Pharmacology and Toxicology, State University of New York, Buffalo, NY; [4]Faculty of Medicine, Menoufia University, Shebin El-Kom, Egypt and [5]National Liver Institute, Menoufia University, Shebin El-Kom, Egypt; [6]Occupational and Environmental Health, University of Iowa, Iowa City, IA.*

Exposure to environmental chemicals, including organophosphorus pesticides, has been associated with attention disorders such as attention deficit hyperactivity disorder (ADHD). Previous studies have identified associations between ADHD and prenatal exposures; additionally one cross-sectional study reported an association between organophosphate metabolite levels and a diagnosis of ADHD among school-age children. However, the impact of occupational pesticide exposure and ADHD in adolescents has not been examined. Adolescent pesticide applicators and non-applicators, 12-18 years old, participated in a 10-month longitudinal study examining health effects from pesticide exposure before, during and after the application season. Parents from a subset of the cohort (N=64) completed the ADHD Rating Scale. Repeated urine and blood samples were collected at various time points during the 10-months to assess biomarkers of exposure and effect. Cumulative urinary TCPy over the study period was used to classify participants into low (< median) and high (≥ median) exposure groups. Participants in the high exposure group had significantly more symptoms of ADHD than participants in the low exposure group. The prevalence odds ratio of having symptoms greater than the cut off in the high exposure group was 2.8 (95% CI = 0.5-14.7) times the odds in the low exposure group. More participants with symptoms above the cut-off score reported applying pesticides at home and had greater cumulative TCPy levels, than participants with symptoms below the cut-off score. However, there were no differences between the groups on years worked as a pesticide applicator for the Ministry of Agriculture. Although limited by a small sample size, this study provides preliminary evidence of an association between occupational pesticide exposure and ADHD symptoms, however, additional research is warranted.

2402 Organophosphorous Pesticide Exposure and Neurobehavioral Performance Among Adolescents in Egypt: A Ten-Month Prospective Study

A. A. Ismail[1], G. Abdel Rasoul[5], M. R. Bonner[3], O. Hendy[5], K. Mara[1], K. Wang[1], J. R. Olson[3] and D. S. Rohlman[1]. *[1]Biostatistics, University of Iowa, Iowa City, IA; [2]Department of Epidemiology and Environmental Health, State University of New York, Buffalo, NY; [3]Department of Pharmacology and Toxicology, State University of New York, Buffalo, NY; [4]Faculty of Medicine, Menoufia University, Shebin El-Kom, Egypt and [5]National Liver Institute, Menoufia University, Shebin El-Kom, Egypt; [6]Occupational and Environmental Health, University of Iowa, Iowa City, IA.*

Chlorpyrifos, an organophosphorus pesticide, is widely used for agricultural operations. The goal of the current study was to examine the impact of chlorpyrifos exposure on biomarkers of exposure and neurobehavioral performance in adolescents before, during and after the application season. At each test session, participants (N=89) completed a neurobehavioral test battery and urine was collected for analysis of the chlorpyrifos metabolite 3,5,6-trichloro-2 pyridinol (TCPy) (biomarker of exposure). Cumulative urinary TCPy over the study period was used to classify participants into low (< median) and high (≥ median) exposure groups. Urinary TCPy increased during the application season with recovery following the end of application. Participants in the high exposure group had significantly elevated metabolite levels throughout the 10-month study period. Deficits in motor skills and slower reaction times, along with deficits in executive function and short-term memory were found between the high and low exposure groups. Changes in neurobehavioral performance across the application season indicate a pattern of impaired performance in the high exposure group compared to the low exposure group. Deficits increased during the application season and remained for months after application ended. This study is the first to examine the impact of changes in pesticide exposure and neurobehavioral performance before, during and after the application season. The findings indicate that neurobehavioral deficits increase during the application season, as exposure also increases, and remain after the application ends, even when the biomarkers of exposure are reduced. This is particularly important when considering the developmental changes that occur during adolescence.

2403 Multi-endpoint Analysis of Organophosphate Developmental Neurotoxicity in a Freshwater Planarian

D. Hagstrom, S. Zhang, H. Hirokawa, P. Taylor and E.-M. S. Collins. *University of California, San Diego, La Jolla, CA.*

Organophosphates (OPs) are among the most commonly used pesticides in the US and act by irreversibly inhibiting acetylcholinesterase (AChE), leading to cholinergic overstimulation due to increased synaptic levels of acetylcholine, paralysis and death. Their environmental abundance, although below acutely toxic levels to humans, has been suggested to be at least partially responsible for the observed increase in childhood neurodevelopmental disorders. However, it is unknown through which mechanisms chronic, low-dose prenatal and infant OP exposure causes neurotoxicity in the developing brain. In this study, we analyzed the toxicological profiles of five common OPs (chlorpyrifos, dichlorvos, diazinon, malathion and parathion) using a multi-endpoint planarian screening platform. The freshwater planarian, Dugesia japonica, is an excellent *in vivo* model for developmental neurotoxicology studies because its remarkable regenerative capabilities allows development to be induced at will by amputation. Moreover, because of their short development time (12 days) and similar size, regenerating and adult animals can be tested simultaneously using the same assays to identify development-specific toxicity. Importantly, despite its simplicity, features of the planarian brain are conserved in the mammalian brain on the molecular and structural levels making mechanistic analysis of neurotoxicity in this animal directly relevant to human health. With this platform, we characterized effects on lethality, regeneration and a range of stimulated and unstimulated behaviors. We found that the different OPs varied widely in toxic dose, affected endpoints and developmental sensitivity. Lastly, through biochemical analysis of AChE activity in OP-treated animals, we found that, depending on the OP, prolonged inhibition of AChE was not necessary for or necessarily indicative of toxic effects. This indicates that toxicity may be induced by alternative mechanisms, necessitating further in depth mechanistic studies.

2404 Kinetic Analysis of the Interactions Between the Organophosphorus Pesticide Metabolite Phorate Oxon and Oximes with Acetylcholinesterase

R. A. Moyer[1], K. G. McGarry[1], M. C. Babin[1], G. E. Platoff[3], D. A. Jett[3] and D. T. Yeung[3]. *[1]Life Sciences Research, Battelle, Columbus, OH; [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD and [3]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Rockville, MD.*

Phorate is an agricultural pesticide that is currently used in the US. Like many other organophosphorus (OP) pesticides, the primary mechanism of the acute toxicity of phorate is acetylcholinesterase (AChE) inhibition mediated by its bioactivated oxon metabolite. Reactivation of inhibited AChE with oximes is a critical aspect of the therapeutic strategy for acute OP intoxication and little is known about the oxime-assisted reactivation of AChE inhibited by phorate oxon (PHO). Thus, phorate represents a widely used pesticide that is highly toxic to mammals with little published data regarding its toxic effects or response to therapy. To help fill this knowledge gap, we evaluated the kinetics of inhibition, reactivation, and aging with PHO, recombinant AChE derived from three species (rat, guinea pig and human), and five oximes: 2-PAM Cl, HI-6 DMS, obidoxime Cl2, MMB4-DMS, and HLö7 DMS. The inhibition rate constants (ki) for PHO were calculated for AChE derived from each species and found to be low (i.e., 4.8 x 103 to 1.4 x 104 M-1 min-1) compared to many other OPs. Obidoxime Cl2 was the most effective reactivator tested and guinea pig AChE was more resistant to reactivation in general than rat or human AChE. Furthermore, the aging rate of PHO-inhibited AChE was very slow (limited aging was observed out to 48 hours) for all three species. The results of this study support the following conclusions: (1) Obidoxime Cl2 was a more effective reactivator than the other oximes tested. (2) The oxime currently approved for use in the US, 2-PAM Cl, showed limited effectiveness in reactivating PHO-inhibited AChE, which suggests that it may have limited usefulness in the clinical management of acute PHO intoxication. (3) The therapeutic window for oxime administration following exposure to phorate (or PHO) is not limited by aging.

2405 Early Differential Induction of Jun-D in the Central Nervous System of Hens Treated with Diisopropylphosphorofluoridate (DFP) May Be Involved in OPIDN

T. V. Damodaran[1,2] and M. B. Abou-Donia[3]. [1]*Biological and Biomedical sciences, North Carolina Central University, Durham, NC;* [2]*Pharmacology and Cancer Biology, Duke University Medical Center, Durahm, NC and* [3]*Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.* Sponsor: T. Damodaran.

Diisopropyl phosphorofluoridate (DFP) produces organophosphorus-ester induced delayed neurotoxicity (OPIDN) in sensitive species including humans. We studied the effect of a single dose of DFP (1.7 mg/kg/sc) on the mRNA expression of Jun D, which is one of the heterodimerizing ITFs (Inducible Transcriptional Factors) of the AP-1 family. The hens were sacrificed at different time points ie 0.25, .0.50, 1 and 2 hrs. Total RNA was extracted from the following brain regions: cerebrum, cerebellum, mid brain, brainstem, and spinal cord. Northern blots prepared using standard protocols were hybridized with Jun D as well as beta-actin and 18S RNA cDNA (control) probes. The results indicate differential regulation of Jun D levels which may be due to the activation of both cholinergic and non-cholinergic pathways of CNS. In the highly susceptible tissues like brainstem and spinal cord, Jun D transcript levels increased at 15 minutes and continued to increase gradually till it reached the maximum at 2 hrs. Overall brainstem showed the maximum induction levels for Jun D induction (302%) mRNA, followed by spinal cord (225%), at 2 hrs time point of all the CNS tissues. Moderately susceptible tissue:cerebellum showed an induction level of 204% at 2 hrs. The induction pattern of mid brain was very similar to that of brainstem. Cerebrum (non-susceptible tissue) did not show any changes in the Jun D levels. The significant increase in Jun D levels along with our earlier observation on the increased c-fos levels and c-jun indicate that AP-1 family of genes may be one of the IEGs involved in the long term changes which eventually lead to OPIDN

2406 Antioxidant and Neuroprotective Effects of AEOL 10150 in a Rat Organophosphate Model

L.-P. Liang[2], P. B. McElroy[2], J. Huang[1], B. J. Day[2] and M. Patel[2]. [1]*Medicine, National Jewish Health, Denver, CO and* [2]*Pharmaceutical Science, University of Colorado Denver, Aurora, CO.*

Prolonged seizure activity or status epilepticus (SE) is one of most common manifestations of organophosphate (OP) exposure which causes brain injury. Previous studies in our laboratory have demonstrated that oxidative stress is a critical mediator of SE-induced neuronal injury. The goal of this study was to determine if diisopropylflurorphoshate (DFP) exposure to rats resulted in oxidative stress and whether scavenging reactive oxygen species (ROS) was neuroprotective against DFP toxicity. DFP treatment increased oxidative stress (glutathione disulfide/glutathione and 3-nitrotyrosine/tyrosine ratios) in a time-dependent manner (i.e. 24h and 48h, but not 6h or 12h) in specific brain regions (i.e. hippocampus and piriform cortex, but not frontal cortex). Neuronal loss measured by Fluoro-Jade B staining was significantly increased in the hippocampus, piriform cortex and amygdala following DFP. One novel and efficacious strategy of neuroprotective countermeasure against OP toxicity is to scavenge ROS using a catalytic antioxidant such as AEOL10150, a broad spectrum manganese porphyrin. Optimal dosing of AEOL10150 was determined by measuring its pharmacokinetics in the presence or absence of DFP. A therapeutic window of AEOL10150 was established in a post-treatment paradigm after DFP treatment which suggested that AEOL10150 treatment 15 min after DFP exposure was sufficient to exert neuroprotection. This study suggests that catalytic antioxidant treatment may be a useful as a novel therapy for OP exposure. This research work was supported by U01NS083422 (M.P.).

2407 A Small Molecule Screen in Stem Cell-Derived Motor Neurons Identifies Inositol as a Potent Neuroprotective Drug Against Organophosphate Neurotoxicity

M. Prissette[2], H. Li[2], T. Obis[2], S. Merwin[2], N. Lamas[1], A. Sharma[2], S. Przedborski[2], C. E. Henderson[2] and D. B. Re[2]. [1]*Project ALS Laboratory, New York, NY and* [2]*Environmental Health Sciences, Columbia University, New York, NY;* [3]*Genome Center HTS Facility, Columbia University, New York, NY;* [4]*Pathology and Cell Biology, Columbia University, New York, NY.* Sponsor: J. Graziano.

Organophosphates (OPs) are a class of pesticides and chemical warfare agents responsible for one million cases of poisoning every year leading to 100,000 deaths. Survivors of OP poisoning suffer from both immediate and delayed neurotoxicity, such as the debilitating paralytic disorder called OP-induced delayed neuropathy (OPIDN). Acute or "cholinergic" neurotoxicity results from the irreversible inhibition of acetylcholine esterase (AchE). In contrast, OPIDN was originally attributed to neuropathy target esterase (NTE) inactivation by a subset of OPs. Yet, accumulating evidence has challenged this hypothesis. Although peripheral axons and spinal motor neurons (MNs) are a target of OPIDN, OP exposure has never been investigated on these cells. Here, we report a mouse embryonic stem (ES) cell-based assay to study OP neurotoxicity to MNs. At doses relevant to acute poisoning and far beyond those necessary for full AchE inhibition, we found that OPs, regardless of their potency to inhibit NTE, are dose-dependently toxic to ES-MNs and their neurites over 14 days. Remarkably, MNs are preferentially vulnerable as compared to other neurons, and rodent primary- and human ES-MNs exhibit a similar vulnerability to OPs. Thanks to ES-MN expandability, we have screened 3,120 compounds against OP neurotoxicity to identify potential neuroprotective therapies. Strikingly, among 6 confirmed hits, we found that inositol is the most neuroprotective molecule for ES-MNs and their processes. This study validates the use of ES-derived neurons for high-scale and reproducible neurotoxicity testing which is not accessible through primary neuronal cultures. Now, further testing in animal models is needed to confirm whether an over-the-counter supplement may offer some clinical translational hope against OPIDN.

2408 Efficacy of Butyrylcholinesterase as a Topical Scavenger Agent Against the Organophosphate Paraoxon

K. Jorge[1], K. Fuhrman[2], J. Hinckley[2] and M. Ehrich[2]. [1]*Midwestern University College of Veterinary Medicine, Glendale, AZ and* [2]*Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA.*

Paraoxon (POX) is an organophosphate that can be used as a surrogate for nerve agents due to its high potency and steep dose response curve. The phosphorylation of acetylcholinesterase (AChE) from POX leads to the buildup of acetylcholine creating cholinergic toxicity and overstimulation of cholinergic receptors (Pope, 1999). Butyrylcholinesterase (BChE) is an esterase that protects from OP toxicity as a catalytic scavenger and helps detoxify insecticides like paraoxon (Saxena, 2002). The ability for a BChE topical agent to protect against POX before and after exposure was determined through observation of motility and gait in mice. All substrates were in 50% ethanol and administered topically as 10 microliter doses. Mice receiving BChE were dosed from a solution of 0.1mg/ml and mice receiving POX were dosed from a solution of 8.33mg/ml. Experimental mice groups (male, CD-1) are as follows: Group 1 (vehicle control, n=10) 50% ethanol; Group 2 (positive control, n=10) POX; Group 3a (n=5) POX 15 minutes before BChE; Group 3b (n=5) POX and BChE simultaneously; Group 4a (n=5) BChE 5 minutes before POX; Group 4b (n=5) BChE 15 minutes before POX. Mice were sacrificed after 80 minutes of observation and whole blood, skin, and brain tissue samples were collected. AChE activity was determined from the samples. AChE activity levels in the brain and blood were not significantly different in mice from group 2 in comparison with groups 3 and 4. However, group 4a showed a significant delay in changes of posture and palpebral closure by 10 minutes and less severity in changes of gait when compared to group 2. This may indicate that clinical symptoms could be delayed by prophylactic treatment of topical BChE 5 minutes before OP exposure. (Funded by NIH as part of the Summer Veterinary Student Research Program at Virginia Maryland College of Veterinary Medicine.)

2409 Quantitative Magnetic Resonance Imaging (MRI) of Brain Lesions Predicts Cognitive Impairment Following Acute Organophosphate (OP) Intoxication in Rats

B. Hobson[4], D. Rowland[4], K. Dhakal[4], D. Bruun[4], D. Tancredi[4], S. Cherry[4], J. Garbow[5] and P. Lein[4]. [1]*Biomedical Engineering, University of California, Davis, CA;* [2]*Center for Molecular and Genomic Imaging, University of California, Davis, CA;* [3]*Molecular Biosciences, University of California, Davis, CA;* [4]*Pediatrics, University of California, Davis, CA and* [5]*Radiology, Washington University, St. Louis, MO.*

Current treatments for OP poisoning do not sufficiently protect against progressive neurodegeneration or delayed cognitive impairment, underscoring the need for preclinical models designed to longitudinally monitor novel therapies. We previously reported that brain lesions detected by *in vivo* MRI are highly correlated with histological indices of neuropathology following intoxication with the OP diisopropylfluorophosphate (DFP). The goal of this study was to determine whether MRI metrics of DFP-induced pathology are predictive of behavioral outcomes. Adult male Sprague Dawley rats were treated with pyridostigmine (0.1 mg/kg, im) prior to administration of DFP (4 mg/kg sc), atropine sulfate (2 mg/kg, ip) and 2PAM (25 mg/kg, im), with a subset receiving a benzodiazepine (5 mg/kg diazepam ip or 0.7 mg/kg midazolam im) 45 min post DFP. Animals were imaged on a Bruker 7T MRI at 3, 7, 28, 65, 91, 182 d post-DFP. DFP elicited moderate-to-severe seizure activity in all rats as determined using a modified Racine scale. T2-weighted and diffusion imaging demonstrated abnormalities in the thalamus, hippocampus, and cerebral and piriform cortices that were significantly reduced by post-exposure treatment with benzodiazepine. Quantification of these abnormalities by pixel-wise analysis of the apparent diffusion coefficient was highly correlated with impaired learning and memory as assessed by Pavlovian fear conditioning. These results suggest that assessment of damage and therapeutic rescue by MRI may be a powerful tool for preclinical drug development. Supported by the NIH CounterACT program (NS079202) and predoctoral fellowships to BH from NIH (GM099608), David and Dana Loury Foundation, ARCS Foundation.

2410 Persistent Behavioral Deficits, Neuroinflammation and Oxidative Stress in Rat Model of Acute Diisopropylfluorophosphate (DFP) Intoxication

M. Guignet[1], K. Dhakal[1], B. Hobson[1], D. Bruun[1], K. Streifel[1], J. Silverman[2] and P. Lein[1]. [1]*Molecular Biosciences, University of California, Davis, Davis, CA and* [2]*Psychiatry and Behavioral Sciences, University of California, Davis, Sacramento, CA.*

Current medical countermeasures for acute organophosphate (OP) poisoning are often not effective in preventing persistent behavioral deficits. A major challenge in developing improved therapies is that the mechanisms by which acute OP intoxication changes behavior are not well understood. Therefore, we used a rat model to characterize behavior and neuropathology following acute intoxication with DFP. Adult male Sprague-Dawley rats were treated with pyridostigmine (0.1 mg/kg, im) 30 min prior to administration of DFP (4 mg/kg, sc), followed by atropine sulfate (2 mg/kg, im) and 2PAM (25 mg/kg, im) to rescue animals from peripheral cholinergic symptoms. Separate cohorts of animals were assessed for behavioral and histological changes at 1 and 2 months post-DFP. Animals exposed to DFP demonstrated hyperreactive behavior and spontaneous recurrent seizures over the 2 month observation period. Performance in the elevated plus maze and open field test suggested that DFP reduced anxiety-like behavior without impairing locomotor activity at 1 and 2 months post-exposure. DFP also caused persistent learning and memory deficits as indicated by Pavlovian fear conditioning. Immunohistochemical analysis revealed a significant increase in astrocytic and microglial activation in the hippocampus, and piriform cortex at 1 but not 2 months post-DFP, and preliminary data indicate increased neuronal levels of 3-nitrotyrosine in these brain regions. These data suggest that neuroinflammation and/or oxidative stress may mediate the persistent behavioral changes witnessed following acute DFP exposure, and thus may represent effective therapeutic targets for neuroprotection. Funding: NIH CounterACT program (NS079202); predoctoral fellowships to MG (NIH GM099608, David and Dana Loury Foundation); postdoctoral fellowship to KS (NIH ES024676).

2411 Analysis of Neonicotinoid Insecticides for Developmental Neurotoxicity

L. P. Sheets[4], A. A. Li[2], D. J. Minnema[5], R. H. Collier[1], M. R. Creek[3] and R. C. Peffer[5]. [1]*Landis International, Inc., Valdosta, GA;* [2]*Health Sciences, Exponent, Inc., San Francisco, CA;* [3]*Human Safety, Valent USA Corporation, Dublin, CA;* [4]*Human Safety, Toxicology, Bayer CropScience, Research Triangle Park, NC and* [5]*Product Safety, Syngenta Crop Protection, Inc., Greensboro, NC.*

The neonicotinoid insecticides were evaluated individually and as a class for evidence of developmental neurotoxicity (DNT) in this first comprehensive critical analysis of the literature and previously-unpublished DNT studies that were performed in accordance with EPA guidelines (OCSPP 870.6300). These insecticides have favorable safety profiles due to their preferential affinity for insect vs. mammalian nicotinic receptor (nAChR) subtypes, poor penetration of the mammalian blood-brain barrier and pesticidal efficacy at low application rates. Nevertheless, examination of their DNT potential is warranted due to their insecticidal mode of action and potential exposures associated with agricultural and residential uses. Our review included *in vivo*, *in vitro* and epidemiology studies from the literature that satisfied the inclusion criteria for the subject, as well as guideline-based DNT studies for all six neonicotinoid insecticides (acetamiprid, imidacloprid, thiacloprid, clothianidin, thiamethoxam and dinotefuran) that are registered in global markets. Consistent and objective evaluation of the EPA DNT guideline studies was based on EPA and Health Canada PMRA reviews, as authoritative bodies responsible for hazard identification and risk assessments. The collective information shows findings at high doses associated with generalized systemic toxicity and no consistent pattern of effects linked to DNT among the six neonicotinoids or for any of these insecticides compared with nicotine. These results indicate the neonicotinoid insecticides do not selectively affect the developing nervous system. This outcome is consistent with the low activity of neonicotinoid insecticides toward mammalian nAChR subtypes and the absence of evidence to suggest these compounds would be developmental neurotoxicants.

2412 Dietary Exposure to the Organochlorine Pesticide Dieldrin Results in Neurotoxic and Cardiotoxic Effects in Zebrafish (*Danio rerio*)

A. M. Cowie[3], L. Slade[3], K. Sarty[1], A. Mercer[2], J. Koh[5], K. Kidd[2], P. C. Kienesberger[3], T. Pulinilkunnil[3] and C. J. Martyniuk[5]. [1]*Biology, University of New Brunswick, New Brunswick, NB, Canada;* [2]*Biology, University of New Brunswick, Saint John, NB, Canada;* [3]*Department of Biochemistry and Molecular Biology, Dalhousie University, Saint John, NB, Canada;* [4]*ICBR, University of Florida, Gainesville, FL and* [5]*Physiological Sciences, University of Florida, Gainesville, FL.* Sponsor: N. Denslow.

Dieldrin (DLD) is an organochlorine pesticide that persists in the environment, despite being discontinued from use for decades. DLD is epidemiologically associated with increased risks of neurodegenerative disease such as Parkinson's disease and is an example of a long lived chemical that can affect human health at low, persistent doses. For these reasons, DLD remains a human health issue and is ranked 18th on the 2013 US ATSDR (4th pesticide). The objective was to determine the effects of dietary DLD exposure in the hypothalamus and the heart of zebrafish (*Danio rerio*). Zebrafish were fed pellets containing 0.03, 0.15, or 1.8 µg/g DLD for 21 days. These doses are environmentally-relevant and result in body burden levels that are comparable to those detected in human blood in epidemiological studies. Transcriptomic profiling determined that DLD had an immunosuppressive effect in the hypothalamus. Gene networks related to mitochondria were also down-regulated, suggesting that mitochondrial function is impaired by DLD. Using iTRAQ proteomics, we quantified ~4000 proteins in the hypothalamus; 226 were differentially expressed in one or more of the DLD treatments and these were related to diseases that included PD. In the heart, DLD affected gene networks related to protein degradation, and genes related to aortic disease, cardiac arrest and ventricular fibrillation were induced by DLD. Given that lysosomal function was altered by DLD at the gene level and three lysosomal v-ATPase protiens were decreased in abundance, we examined the pathway which regulates the lysosome (Akt/mTOR). Immunoblotting for mTOR (mechanistic target of rapamycin) revealed hyper-phosphorylation of this protein in the heart, and Akt/protein kinase b, an upstream activator of mTOR, showed increased phosphorylation at serine 473 with DLD, suggesting there is activation of this pathway following DLD treatment. These data suggest that DLD may not only be associated with neurodegeneration, but also cardiovascular disease.

2413 Deltamethrin Exposure Inhibits Adult Hippocampal Neurogenesis and Causes Deficits in Learning and Memory in Mice

M. M. Hossain and J. R. Richardson. *Pharmaceutical Sciences, Northeast Ohio Medical University, Rootstown, OH.*

Deficits in learning and memory are often associated with disruption of hippocampal neurogenesis, which is regulated by numerous processes, including precursor cell proliferation, survival, migration, and differentiation to mature neurons. Recent studies demonstrate that adult born neurons in the dentate gyrus (DG) in the hippocampus can functionally integrate into the existing neuronal circuitry and contribute to hippocampal-dependent learning and memory. Recently, we reported that repeated adult exposure to deltamethrin (3 mg/kg every 3 days for 2 months) resulted in reduction of progenitor cell proliferation in the DG of hippocampus and learning deficits in mice. Here, we investigated whether similar effects occur at an earlier time-point. Deltamethrin exposure significantly decreased bromodeoxyuridine (BrdU)-positive cells (39%) in the DG of the hippocampus, indicating decreased cellular proliferation. In addition, deltamethrin-treated mice exhibited a decrease in nestin-expressing neural progenitor cells (44%) and a 33% reduction in the expression of doublecortin (DCX), an early neuronal differentiation marker. We also found that deltamethrin exposure resulted in an increase of BrdU/caspase-3 co-labeled cells (25%) and a decrease of BrdU/NeuN-positive cells (37%), suggesting potential inhibition of neuronal differentiation and cell survival. Finally, these changes were accompanied by deficits in two hippocampal-dependent behavioral tests, the novel object recognition task and Morris water maze. These findings indicate that relatively short-term exposure to deltamethrin causes significant deficits in hippocampal neurogenesis that is associated with impaired learning and memory.

2414 Effect of Repeated Juvenile Exposure to Permethrin and Deltamethrin on the Performance of Adolescent Rats in the Elevated Plus Maze

N. Alugubelly, A. N. Mohammed and R. L. Carr. *Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS.*

Pyrethroid insecticides are frequently used for insect control in both households and in agriculture. Because of the widespread use, there is concern that children are more at risk to the negative effects of the pyrethroids because of their ongoing nervous system development. Some studies have linked developmental exposure to pyrethroid insecticides to greater incidence of attention deficit/hyperactivity disorder (ADHD) in children. The goal of the present study was to investigate the effects of developmental exposure to permethrin, a Type I pyrethroid, and deltamethrin, a Type II pyrethroid, on anxiety-related behaviors in adolescence. Ten day old rat pups were exposed orally to either corn oil, 25 mg/kg permethrin, or 0.25 mg/kg deltamethrin daily for 7 days. On day 29, male and female rat pups were tested in an elevated plus maze. The rats were naive to the testing room and the apparatus and the testing was conducted under high levels of light (~700 lux). Female rats exposed to both permethrin and deltamethrin had significantly higher percent open arm entries and percent time spent in the open arms as compared to controls. No significant effects on total entries or closed arm entries were present indicating that the changes were not to an increased level of activity. These data suggest that juvenile exposure to pyrethroids can result in increased anxiolytic-like behavior in adolescent females. No significant effects were observed in male rats suggesting that these effects are sex-specific.

2415 Development of an *In Vitro* Blood-Brain Barrier Model to Measure the Transendothelial Electrical Resistance of Tight Junctions Due to Exposure to Pyrethroids

K. Flaugher, A. Keely and M. Chan. *Southern Illinois University Edwardsville, Edwardsville, IL.*

Pyrethroids have been used widely inside the home in aerosol cans, insecticidal pet shampoos, insecticide bombs, mosquito repellents, and treatments for lice applied directly to humans. They act primarily on the nervous system by interfering with the transmission of neural impulses via action on sodium channels to cause neurotoxicity. The toxicities of pyrethroids are different, depending on the absence or presence of the alpha-cyano group. The human brain microvascular endothelial cells (HBMECs) were used to develop a model to study the effects of pyrethroids on permeability of tight junctions in the blood-brain barrier (BBB). We hypothesized that the BBB permeability, measured as transendothelial electrical resistance (TEER) and FITC-dextran flux decreased in a dose- and time-dependent manner when monolayers were treated with pyrethroids. We used bifenthrin (Type I) and deltamethrin (Type II) as our test chemicals to illustrate the difference in responses between these two types of pyrethroids. Cytotoxicity testing revealed that deltamethrin and bifenthrin did not cause cell death at concentrations of 10 micro Molar and below. Our TEER and FITC-dextran permeability results showed that bifenthrin and deltamethrin decreased in a dose- and time-dependent manner when monolayers were treated with these pyrethroids at concentrations of 0.01 micro Molar to 10 micro Molar. These data suggest that the effect of deltamethrin on the tight junctions of the BBB model was more significant compared to bifenthrin because of the presence of the alpha-cyano group. This data will be useful when incorporated into the quantitative extrapolation of *in vitro* to *in vivo* model.

2416 Fc Gamma Receptors Are Expressed in the Developing Rat Brain and Activate Downstream Signaling upon Cross-Linking with Immune Complex

M. Stamou[1,2], A.-C. Grodzki[2] and P. J. Lein[2]. [1]*Health Science and Technology, Swiss Federal Institute of Technology (ETH) Zurich, Zurich, Switzerland and* [2]*Molecular Biosciences, University of California Davis, Davis, CA.*

Exposure of the developing brain to immune mediators, including antibodies, is postulated to increase risk for neurodevelopmental disorders and neurodegenerative disease. It has been suggested that immunoglobulin G-immune complexes (IgG-IC) activate Fc gamma receptors (FcgR) expressed on neurons to modify signaling events in these cells. However, testing this hypothesis is hindered by a paucity of data regarding neuronal FcgR expression and function. Similarly, little is known about FcgR expression by astrocytes. To address these data gaps, we assessed FcgR expression on neurons and astrocytes from the developing rat brain and investigated whether their regulation and signaling was similar to that of FcgR in immune cells. As determined using qPCR, Fcgrla, FcgrIIa, FcgrIIb, FcgrIIIa and Fcgrt transcripts were detected in the rat cortex, hippocampus and cerebellum at postnatal days 1 and 7, and in primary hippocampal and cortical cell cultures obtained from neonatal rats. *In vitro* Fcgr expression was modulated by IFNγ. Western blotting detected FcgRIa and FcgRIIIa in cortical cell lysates, and immunocytochemistry confirmed expression of FcgRIa and FcgRIIb in hippocampal and cortical neurons and astrocytes. *In vitro* exposure to IgG-IC increased [Ca2+]i and Erk phosphorylation and triggered FcgR internalization. These data demonstrate that neurons and astrocytes of the developing hippocampus and cortex express FcgR, and that exposure of these cells to IgG-IC triggers downstream events associated with canonical FcgR signaling. These findings suggest a novel mechanism by which immunoglobulins modulate signaling in the developing brain and potentially influence vulnerability to neurodevelopmental disease. This work was supported by the NIH (grants ES014901, ES011269 and ES04699) and the US EPA (grant R833292).

2417 5, 6-Benzoflavone Exposure Alters Lipogenic Gene Expression and Lipid Synthesis during Lactation

K. Belton, L. Zhang, G. H. Perdew and A. Patterson. *Penn State University, University Park, PA.*

Lactation creates continuous demand making the mammary gland (MG) one of the most active sites of triglyceride synthesis in the body. Milk lipids are composed of 98% triglycerides, 1% phospholipid, and 0.5% cholesterol and cholesterol esters. At present few details exist about how dioxin and dioxin-like compounds (DLCs) affect lipid synthesis processes within the lactating MG. This study highlights that inappropriate exposure to 5, 6-benzoflavone (BNF) an AHR (aryl hydrocarbon receptor) ligand can severely interrupt lipid synthesis as well as MG differentiation. Data demonstrates that BNF exposure significantly decreases mRNA expression of sterol regulatory element-binding protein 1C and fatty acid synthase in mice MG and HC-11 cells (both cholesterol and lipid synthesis genes are regulated by this transcription factor family (i.e., SREBP1/2)). BNF caused significant decreases in triglyceride production in HC-11 cells compared to control. Seeing as millions of mothers annually have significant difficulty initialing breast-feeding, deciphering toxicological factors that affect the ability of the mammary gland function would expectedly heighten the awareness of the potential contaminates.

2418 Nuclear Receptor 4A1 Is a Drug Target for Treating Rhabdomyosarcoma

A. Lacey[2], E. Hedrick[2] and S. Safe[1]. [1]*Institute of Biosciences & Technology, Texas A&M Health Science Center, Houston, TX and* [2]*Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.*

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma primarily observed in children/adolescents and accounts for 5% of all pediatric cancers and 50% of soft tissue sarcomas in children. Some RMS patients are successfully treated with surgery and cytotoxic drug combinations, resulting in adverse health effects. Therefore, the development of relatively non-toxic, RMS mechanism-based agents is essential. Data mining of RMS patient array data indicates that the orphan receptor, NR4A1, is highly expressed in some tumors and RMS cell lines, including RD and RH30 cells, which represent embryonal and alveolar RMS tumors, respectively. The function of NR4A1 in RMS, investigated by RNA interference (RNAi/siNR4A1),significantly decreased cell growth and induced apoptosis as shown by increased Annexin V staining. Recent studies in this lab have shown that among a series of 1,1-bis (3'-indoyl)-1-(p-substituted phenyl) methane (C-DIM) analogs, the p-hydroxyphenyl (DIM-C-pPhOH) and p-carboxymethylphenyl (DIM-C-pPhCO2Me) analogs bind the ligand binding domain of NR4A1 and exhibit NR4A1 antagonist activity. Treatment of RD and SJCRH30 cells with DIM-C-pPhOH and DIM-C-pPhCO2Me inhibited cell growth and induced apoptosis; these effects were accompanied by decreased expression of several specificity protein (Sp1)-regulated genes, including EGFR, Bcl-2, and cyclin D1. NR4A1 antagonists also decreased expression of thioredoxin domain-containing 5 (TXNDC5) and isocitrate dehydrogenase 1 (IDH1), resulting in oxidative and endoplasmic reticulum (ER) stress. This oxidative stress also induced sestrin2, which activated AMPKα to inhibit mTOR signaling. Thus NR4A1 regulated growth promoting/prosurvival pathways in RMS cells, similar to those previously observed for other solid tumor-derived cell lines and NR4A1 antagonists inhibited these prooncogenic pathways by inhibiting expression of selected NR4A1 regulated genes. We have also observed that NR4A1 regulates migration/invasion of RMS cells and current studies are focued on genes required for these responses and the effectiveness of NR4A1 antagonists as a novel class of drugs for cancer chemotherapy.

2419 Divergent Evolution of the Human Ah Receptor from Neanderthal Conferred Enhanced Resistance to Fire Use

T. D. Hubbard[2], I. A. Murray[2], W. H. Bisson[1] and G. H. Perdew[2]. [1]*Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR and* [2]*Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA.*

The habitual use of fire supported the socio-cultural evolution of Homo sapiens by conferring a source of light, consistent warm temperatures, and more energetically available food sources. However, incomplete combustion of organic materials, such as wood, causes production of polycyclic aromatic hydrocarbons (PAHs) resulting in increased exposure to ligands for the aryl hydrocarbon receptor (AHR) and their associated toxicities. Comparative studies have shown that the human AHR exhibits reduced binding capacity with regard to prototypical PAH agonists when compared to rodent homologues. Such differences suggest selective adaptation may have arisen during the course of mammalian evolution. Here, we present evidence supporting the hypothesis that this selective desensitization of the human AHR to PAHs arose during or after speciation of *H. sapiens* and that exposure to high concentrations of PAHs, as a consequence of Paleolithic fire use, provided selective pressure for diminished ligand binding. Interspecies AHR analyses, through utilization of ligand binding assay, DNA binding assay, and AHR-dependent gene expression, identified a single amino acid substitution (A381V) as the determinant in reduced PAH affinity exhibited by *H. sapiens* relative to their *Homo neanderthalensis* and primate counterparts. Notably, *H. sapiens* AHR responsiveness to endogenous ligands derived from tryptophan and indole metabolism remains unperturbed. These data suggest differential AHR responsiveness to PAHs as a contributing factor to the supplantation of *H. neanderthalensis* by *H. sapiens*. This project is supported by NIH grants ES004869 and ES019964.

2420 Metagenomics Analysis of the Mouse Gut Microbiota Following Exposure to the Environmental Contaminant and AHR Agonist 2,3,7,8- Tetrachlorodibenzofuran

R. Nichols, L. Zhang, P. B. Smith, G. H. Perdew and A. Patterson. *Penn State University, University Park, PA.* Sponsor: A. Patterson.

The scientific community is increasingly appreciative of the important role the aryl hydrocarbon receptor (AHR) plays in modulating the host-gut microbiota metabolic axis. Emerging data suggests that the gut microbiota are an important component in the AHR-mediated response to persistent environmental contaminants including 2,3,7,8-tetrachlorodibenzofuran (TCDF). However how chemicals like TCDF impact the gut microbiota (population and function) has not been adequately addressed. In this study, wild-type male C57Bl/6J mice were exposed to 24 ug/kg (p.o.) TCDF for five days and cecal extracts analyzed using illumina Hiseq metagenomic analysis. The whole genome sequencing data analysis (done with the Humann2 toolkit) revealed significant changes in the gut microbiota including a decrease in several important pathways (coenzyme A biosynthesis, starch degradation, glycolysis and adenosine biosynthesis) following exposure to TCDF. Further, TCDF exposed gut microbiota had an increase in purine degradation and legion-aminate biosynthesis. The metagenomics results were validated with metabolomics data obtained by a combination of mass spectrometry- and 1H nuclear magnetic resonance spectroscopy analyses of the cecal microbiota. In summary, exposure to environmental contaminants including TCDF significantly impact the gut microbiota.

2421 Examining Role of Peroxisome Proliferator-Activated Receptor-β/δ (PPARβ/δ) in Colon Cancer

X. Wang. *Center for Molecular Toxicology and Carcinogenesis, Penn State University, University Park, PA.* Sponsor: J. Peters.

The role of PPARβ/δ in colon cancer is controversial. The APC and/or CTNNB1 genes are mutated in over 80% of colon cancer in humans. The first study to report a role between colon cancer and PPARβ/δ suggested that PPARβ/δ was a target of the APC/β-CATENIN pathway and promoted tumorigenesis by enhancing cell proliferation of mutant colon cancer cells. By contrast, subsequent studies do not support this hypothetical pathway, as expression of CYCLIN D1 is higher in human colon cancer cell lines with mutant APC and/or CTNNB1 whereas expression of PPARβ/δ protein was unchanged as compared to controls with wild-type APC and CTNNB1. Further, expression of PPARβ/δ was also lower in human and mouse colon tumors as compared to control tissue. To further evaluate the role of PPARβ/δ in colon cancer, the present study examined whether differences in mutant APC and/or CTNNB1 caused differences in the nuclear localization of PPARβ/δ in human colon cancer cell lines. Additionally, these studies also determined whether mutant APC in a human colon cancer cell line (DLD1) caused differences in ectopic xenografts as compared to a human colon cancer cell line with wild-type APC (RKO). The effect of over-expressing PPARβ/δ and/or ligand activation of PPARβ/δ in the latter model were also examined. Interestingly, expression of CYCLIN D1 was higher in the cytosol in human colon cancer cells with mutant APC and/or CTNNB1 as compared to controls. By contrast, no change in the nuclear or cytosolic localization of PPARβ/δ was observed between any of the human colon cancer cell lines. Over-expression of PPARβ/δ and/or ligand activation of PPARβ/δ inhibited growth of ectopic xenografts derived from either RKO or DLD1 cells. No significant differences in tumorigenicity was observed between the two human colon cancer cell lines, despite the fact that DLD1 cells have a mutant APC gene. Results from these studies indicate that PPARβ/δ is not upregulated or shuttled to the nucleus when APC and/or CTNNB1 genes are mutated. Further, results from these studies indicate that over-expression and/or ligand activation of PPARβ/δ inhibits ectopic xenograft growth, independent of APC mutational status. (Supported by CA124533, CA140369).

2422 Cellular Signaling Pathways Affected by TCDD in Laser Capture Microdissected Centrilobular and Periportal Hepatocytes Supports Regional Specificity for Key Events in the Adverse Outcome Pathway for AHR-Mediated Rodent Liver Tumor Promotion

J. Harrill[2], B. Parks[2], M. Black[2], P. McMullen[2], R. Budinsky[1], J. C. Rowlands[1] and R. Thomas[2]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Sustained activation of the aryl hydrocarbon receptor (AHR) is the molecular initiating event in the adverse outcome pathway (AOP) for rodent liver tumor promotion by certain AHR ligands. Intermediate key events preceding hepatocellular and bile duct tumors in rodents include changes in cellular homeostasis, inhibition of apoptosis, hepatopathy (with inflammation) and cellular proliferation. It has been hypothesized that key events (KE) in the AOP may display zonal specificity in the liver. AHR knockout (AHR-KO) and wild type (WT) rats (n = 8 rats/dose/genotype) were dosed by oral gavage for 4 weeks with varying concentrations of TCDD in corn oil (0, 3, 22, 100, 300, 1000 ng/kg/day). Centrilobular (CL) and periportal (PP) hepatocytes were collected from liver cryosections using laser capture microdissection. Transcriptomic profiles were obtained using Affymetrix HT RG230PM arrays. Dose-dependent changes in transcript expression were identified in WT CL and PP hepatocytes, but not AHR-KO hepatocytes from either zone. Transcripts with dose-dependent changes in expression were used as input for pathway enrichment analysis (MetaCoreTM). Enrichment of pathways for nuclear receptor signaling, cholesterol biosynthesis and bile acid circulation were observed in both CL and PP hepatocytes. Pathways for inflammation, immune responses, apoptosis and survival were enriched in CL hepatocytes only. Pathways relating to cell cycle and WNT signaling were enriched in PP hepatocytes only. Benchmark dose (BMD) analysis demonstrated that pathways enriched in both cell populations (i.e. nuclear receptor signaling) had lower BMDs than pathways that are consistent with other KEs in the AOP. These findings may provide information for elucidating molecular pathways underlying KEs in response to sustained AHR activation.

2423 Hepatic Aryl Hydrocarbon Receptor Activity Attenuates Fibroblast Growth Factor 21 Induction

N. G. Girer[2], I. A. Murray[2] and G. H. Perdew[2]. [1]*Biochemistry, Microbiology, Molecular Biology, Pennsylvania State University, State College, PA and* [2]*Veterinary and Biomedical Sciences, Pennsylvania State University, State College, PA.*

Fibroblast growth factor 21 (FGF21) is a unique member of the fibroblast growth factor family that has recently garnered much attention for its potential use as a treatment for obesity. Administration of recombinant FGF21 in various models of obesity results in weight loss, fat pad reduction, and improved insulin sensitivity. Physiologically, several transcription factors induce Fgf21, including PPARα (fasting), ChREBP (elevated extracellular glucose) and CREBH (ER stress). Recent evidence suggests that the aryl hydrocarbon receptor (AHR) might also increase Fgf21 expression. AHR is a ligand-activated transcription factor with an established role in mediating TCDD toxicity. Several lines of evidence indicate that AHR also plays an important role in metabolic homeostasis. For example, we previously demonstrated that AHR is able to attenuate hepatic *de novo* cholesterol and fatty acid synthesis. However, the homeostatic role of AHR in the absence of exogenous ligand remains largely unknown. In this study, we examined the role of AHR in Fgf21 regulation and metabolic homeostasis using a hepatocyte-targeted, conditional AHR knockout mouse model (CreAlbAhrFx/Fx). When maintained on a defined diet, CreAlbAhrFx/Fx mice exhibit a 4-fold increase in hepatic Fgf21 expression, concurrent with induction of Fgf21-target gene Igfbp1 and decreased expression of genes involved with fatty acid synthesis. Analysis of the Fgf21 promoter reveals several putative DRE sequences, one of which overlaps binding sequences for transcription factors PPARα, ChREBP, and CREBH. Using EMSA, we confirm that the AHR/ARNT heterodimer is able to bind to this sequence. Utilizing a cell-based reporter assay, we also demonstrate that ligand activation of AHR is able to block the induction of Fgf21 by these transcription factors. Finally, we show that ligand-activated AHR similarly blocks FGF21 transcription in primary human hepatocytes. In summary, we demonstrate that the AHR plays an important role in the attenuation of hepatic Fgf21 expression. We hypothesize that AHR repression of Fgf21 gene expression represents a novel role for the receptor in metabolic homeostasis, in the absence of exogenous ligands. This project is supported by NIH grants ES004869 and ES019964.

2424 Altered Basal and TCDD-induced Gene Expression in AHRRa-Null Zebrafish Embryos Generated by ZFN-Mediated Gene Targeting

M. E. Hahn, N. Aluru, S. I. Karchner and D. G. Franks. *Biology, Woods Hole Oceanographic Institution, Woods Hole, MA.*

The aryl hydrocarbon receptor (AHR) repressor (AHRR), a bHLH-PAS protein, is a transcriptional repressor of AHR and other transcription factors (HIF, ER) and is regulated by an AHR-dependent mechanism. However, the physiological and toxicological roles of AHRR are not well understood. We demonstrated earlier (Jenny et al. 2009 Toxicol. Sci. 110:426) that knockdown of AHRRa (one of two AHRR paralogs) in zebrafish embryos using morpholino anti-sense oligonucleotides results in developmental phenotypes such as pericardial edema, craniofacial malformations, and cardiac deformities, similar to effects caused by exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). AHRRa morphants also exhibit down-regulation of genes associated with photoreceptor development (Aluru et al. 2014 Toxicol. Sci. 139:381). To further assess AHRRa function, we used ZFN (zinc finger nuclease) technology to generate a line of zebrafish with a 7-bp deletion in exon 3 of AHRRa, leading to a truncated AHRR (110 aa, of which 38 are from AHRRa, compared to the 550-aa wild-type (wt) AHRRa). The truncated AHRRa protein, which contains basic regions but lacks the HLH and PAS domains, did not repress AHR *in vitro*, suggesting that it is inactive (null). Unlike AHRRa morphants, untreated AHRRa-null fish did not display a TCDD-like phenotype. Exposure of AHRRa-null embryos to TCDD caused defects similar to those in TCDD-exposed wt embryos. RNA-sequencing revealed that basal expression of 562 genes differed significantly between the null and wt embryos, including down-regulation of genes associated with photoreceptor development, as seen in AHRRa morphants. TCDD-induced gene expression patterns for the prototypic AHR target genes were similar for null and wt embryos. However, 22 genes were differentially regulated by TCDD in the AHRRa-null embryos as compared to wt embryos (>2-fold; 5% FDR). We are currently investigating the link between these differentially expressed genes and the function of AHRRa in development and AHR signaling. [Supported by NIH R01ES006272]

2425 Activation of the Aryl Hydrocarbon Receptor Attenuates the Cholesterol Synthesis Pathway via Alterations in the Sterol Regulatory Element Binding Protein-2 Levels

G. E. Muku, I. A. Murray and G. H. Perdew. *Veterinary and Biomedical Sciences, Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA.*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that has a well-established role in mediating dioxin toxicity. The physiological role of AHR has been the focus of numerous studies in recent years, and it remains a topic of considerable interest. We have previously demonstrated that hepatic expression of cholesterol biosynthetic genes is down-regulated by the activation of AHR in a dioxin response element (DRE)-independent manner. To further investigate the role of AHR in cholesterol synthesis, we examined the influence of functionally distinct classes of AHR ligands (TCDD, SGA360, and SGA315) on the levels of enzymes involved in the biosynthetic pathway of cholesterol. Quantitative PCR and Western blot analyses using the human Caco-2 cell line revealed coordinate repression of both mRNA and protein levels upon ligand activation of AHR for many of the cholesterol biosynthetic enzymes, including; HMGCR, MVD, IDI1, FDPS and FDFT1. Transcription of these enzymes is predominantly regulated by sterol-regulatory element-binding protein-2 (SREBP-2), and this led us to hypothesize that AHR activation suppresses the activity of SREBP-2. Western blot analyses showed a significant decrease in mature nuclear SREBP-2 levels upon ligand treatment. In contrast, the precursor form of SREBP-2 was not affected by AHR activation, which indicates that AHR does not interfere with the proteolytic cleavage of SREBP-2 within the Golgi complex. The selective AHR modulator SGA360 does not induce nuclear AHR translocation, yet facilitates nSREBP-2 turnover, supporting the concept of cytoplasmic AHR activity. These data emphasize a critical role for AHR in the systemic homeostatic regulation of the cholesterol synthesis pathway and suggests that modulating AHR activity might have therapeutic potential. This project is supported by NIH grant ES004869.

2426 Aryl Hydrocarbon Receptor Expression Is Required to Maintain Intestinal Host-Microbe Homeostasis in C57BL6/J Mice

I. A. Murray, L. Zhang, R. Nichols, I. Albert, A. D. Patterson and G. H. Perdew. *Center for Molecular Toxicology & Carcinogenesis, The Pennsylvania State University, University Park, PA.*

The intestinal microbiota is recognized as a major contributor to host physiology, impacting diverse signaling networks not only within the intestinal tract but also holistically. Environmental and genetic factors represent key components in establishing and maintaining the intestinal microbiota. The aryl hydrocarbon receptor (AHR) is emerging as a pleiotropic factor, modulating pathways beyond its established role as a xenobiotic sensor. Recently, it has been shown that the AHR modulates immune surveillance in the intestinal tract by various mechanisms including retention of intraepithelial lymphocytes. As such, environmental or genetic manipulation of AHR activity is likely to influence host-microbe homeostasis. Utilizing cohoused C57BL6/J Ahr-/+ and Ahr-/- littermates, which were then subjected to 14 days of genotypic segregation, we examined the influence of AHR expression on the composition/functionality of the intestinal microbiota together with subsequent alteration of host physiology. 16S rDNA sequencing and quantitative PCR (qPCR) analyses revealed significant changes in phyla abundance, particularly Verrucomicrobia, together with segmented filamentous bacteria, and a marked increase in species diversity, in Ahr-/- following genotypic isolation. Metagenomic analyses indicate that the microbial composition in Ahr-/- is associated with functional changes in bacterial metabolism, aspects of which were confirmed through 1H-NMR metabolomics. qPCR analyses identified Ahr-/--dependent increases in ileal Cd14, Saa1/3 and Lcn2 mRNA, indicative of increased inflammatory tone. Cecal transfer of Ahr-/- microbiota into wild-type (Ahr+/+) germ-free mice recapitulated the increase in Verrucomicrobia and inflammatory tone observed in Ahr-/- mice. These data highlight a pivotal role for the AHR in maintaining microbiota homeostasis and suggest that environmental modulation of AHR activity likely exerts microbe-dependent effects on host physiology. Supported by NIH grants ES004869/ES019964

2427 Agonist-Specific Aryl Hydrocarbon Receptor-Mediated Differential Gene Expression

A. D. Joshi and C. J. Elferink. *Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX.*

The Aryl Hydrocarbon Receptor (AhR) is a ubiquitous, ligand activated basic helix-loop-helix transcription factor of Per/ARNT/Sim family known to regulate adaptive and toxic responses to a variety of chemical pollutants including the polycyclic aromatic hydrocarbons and halogenated aromatic hydrocarbons, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Upon activation by TCDD, the AhR translocates into the nucleus and binds to DNA at the Xenobiotic Response Element (XRE) in partnership with the Aryl hydrocarbon Receptor Nuclear Translocator (ARNT) to drive target gene expression, most notably CYP1A1. We have recently identified stanniocalcin 2 (Stc2) as a novel AhR target gene, and discovered agonist-specific transcriptional responsiveness. We screened various tryptophan catabolites using isolated primary hepatocytes and demonstrated that cinnabarinic acid (CA) and Quinolinic acid (QA) are novel endogenous AhR ligands that specifically induce Stc2, but not CYP1A1. In contrast, CA and QA are unable to induce CYP1A1 expression. Chromatin immunoprecipitation indicates that CA and QA treatment results in recruitment of AhR-ARNT complex to the region of Stc2 promoter harboring an XRE cluster (-450 to -193). Using isolated primary hepatocytes from AhR wild-type and liver specific AhR conditional knockout (AhR-CKO) mice, we demonstrated that AhR activation by CA and QA but not TCDD, induces Stc2. The AhR-dependent Stc2 expression in response to the endogenous agonists confers cytoprotection against ER and oxidative stress induced apoptosis. This indicates that exogenous and endogenous AhR agonists induce dichotomous signaling cascades with distinct physiological consequences. Future studies will characterize this differential responsiveness at a genome-wide level by identifying the binding sites occupied by AhR in response to TCDD and CA/QA exposure, and reconcile AhR-ARNT DNA binding in the chromatin with changes in target gene expression using ChIP-seq and RNA-seq, respectively. In summary, this study describes a novel physiological role for AhR and highlights the complexities and potential biological ramifications resulting from agonist-specific AhR activation.

2428 Loss of Hepatic Aryl Hydrocarbon Receptor Alters the Thermogenic Properties of White Fat and Protects Against Diet Induced Obesity

D. E. Carter[2], C. Porter[2] and C. Elferink[2]. [1]*Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston, TX and* [2]*Surgery, The University of Texas Medical Branch, Galveston, TX.* Sponsor: C. Porter.

The aryl hydrocarbon receptor (AhR) is a cytosolic, ligand activated transcription factor commonly known for its role in xenobiotic metabolism. However, the generation of AhR-null mice has revealed physiological roles for the AhR in lipid metabolism and energy expenditure. The AhR is highly expressed in the liver, which is a major endocrine regulator of both overall lipid and energy homeostasis. To investigate how AhR activity in the mouse liver affects lipid metabolism, we utilized adult female (8-10 weeks) AhR-floxed (AhRfx/fx controls) and liver specific AhR-CKO (AhRfx/fx Alb-CreERT) mice. We discovered a novel phenotype wherein; AhR-CKO mice exhibited significantly reduced body weight gains and increased expression of uncoupling protein 1 (UCP1) in gonadal white adipose tissue (GWAT) depots vs. controls while maintained on a normal chow diet. AhR-CKO mice brown adipose tissue (BAT) and GWAT also revealed significant increases in uncoupled mitochondrial respiration rates compared to controls, consistent with the increased expression of UCP1 the and multilocular morphology observed in H&E sections. These changes are indicative of browning of the white adipose tissue. We also placed mice on a 41 Kcal % high fat diet (HFD) for 3 months, which revealed a resistance to increased weight gain and hepatic steatosis in AhR-CKO vs. controls. Our results demonstrate novel physiological roles for the AhR in the liver in modulating the thermogenic properties of white fat as well as protection against weight gain and fatty liver while on an obesogenic diet.

2429 Regulation of Aquaporin 3 Expression by the Arylhydrocarbon Receptor Pathway Is Critical for Cell Migration

C. Tomkiewicz-Raulet. *Université Paris Descartes, INSERM UMR 1124 Pharmacology, Toxicology and Cell Signaling, Paris, France.* Sponsor: R. Barouki.

The regulation of cell migration is a key factor in the dissemination of metastatic cells during tumor progression. Aquaporins (AQP) are membrane channels which allow transmembrane fluxes of water and glycerol in the cells of a variety of mammalian tissues. We found that AQP3, which has been incriminated in cancer progression, is regulated by the AhR (Arylhydrocarbon or dioxin receptor). AhR is a transcription factor which is activated by environmental pollutants. It has been shown to regulate several cellular processes including cell migration and plasticity. *In vivo*, following exposure to the AhR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the expression of AQP3 was increased significantly in several murine tissues including the liver. *In vitro*, exposure of human liver hepatocellular carcinoma HepG2 cells line to TCDD also increased the expression of AQP3 mRNA and protein. These effects resulted from activation of the AhR as shown by RNA interference, chromatin immunoprecipitation and the use of several ligands specific for the AhR. Immunofluorescence and real-time analysis of cell migration (XCELLigence) demonstrated that the knockdown of AQP3 mRNA using small interfering RNA impairs the remodeling of cell shape and the triggering of cell migration that is induced by TCDD. Our work reveals, for the first time, a link between the exposure to a pollutant and the induction of an aquaporin which is suspected to play a role during cancer metastasis.

2430 Novel Roles for Ahr and Arnt in the Regulation of Alcohol Dehydrogenase Expression in Human Hepatic Cells

E. Attignon[1,2], A. Leblanc[1,2], B. Le-Grand[1,2], C. Duval[1,2], M. Aggerbeck[1,2], H. Rouach[1,2] and E. Blanc[1,2]. [1]*INSERM UMR-S 1124, Paris, France and* [2]*Paris Descartes University, Paris, France.* Sponsor: R. Barouki.

The exposure of humans to environmental pollution has become a major health concern. TCDD (2,3,7,8-Tetrachlorodibenzo-para-dioxin) is a lipophilic xenobiotic which activates the AhR (Aryl hydrocarbon Receptor) signaling pathway. We previously showed, by a genome-wide transcriptome analysis, that the mRNA levels of most of the alcohol metabolism enzymes (cytochrome P450 2E1 or CYP2E1 and the alcohol dehydrogenases ADH1, 4 and 6) were decreased following exposure of an *in vitro* model of differentiated human hepatic cells (HepaRG) to 25 nM TCDD for 30 h. Some ADHs also are known to be involved in retinol metabo-

lism. We now have found that the the ADH and CYP2E1 mRNA levels in HepaRG cells were decreased by a range of concentrations of TCDD (0.1 nM to 25 nM) as rapidly as 8 h after treatment of the cells. The amounts of proteins also were decreased after 72 h of treatment (25 nM TCDD). The half-lives of the ADH proteins were not modified by treatment with TCDD which suggests that the regulation of ADH expression is transcriptional. The AhR antagonist CH-223191 or AhR siRNA reduced the inhibitory effect of 25 nM TCDD on the expression of ADHs. These effects were mediated by the genomic pathway (via the AhR/ARNT complex) and the c-SRC non-genomic pathway was not involved. Exposure of HepaRG cells to other AhR ligands (3-Methylcholanthrene and PCB-126) also resulted in decreased levels of ADH mRNAs. TCDD also was found to regulate the expression of ADH4 in the HepG2 human hepatic cell line, in primary human hepatocytes and in the livers of mice. Activation of the AhR/ARNT signaling pathway by AhR ligands represents a novel mechanism for the regulation of the expression of ADHs. However, the direct link between this pathway and the decrease in the expression of the alcohol metabolizing enzymes is still unknown. Further studies are needed to understand how an inevitable exposure to pollutants, which alters the enzymes of ethanol/retinol metabolism, may be associated with a higher risk of liver diseases and/or alcohol abuse disorders.

PS 2431 Application of the PPARg2-CALUX® Assay to the Biological Detection of Endocrine Active Chemicals

C. Dusserre[2,3], J. Mollergues[3], E. Lo Piparo[3], M. Marin-Kuan[3], H. Besselink[1], B. Schilter[3] and K. C. Fussell[3]. *[1]BioDetection Systems, Amsterdam, Netherlands; [2]Faculte de Pharmacie de Paris, Universite Paris Descartes, Paris, France and [3]Nestle Research Center, Nestec Inc., Lausanne 26, Switzerland.*

Some environmental contaminants, or 'obesogens', are suspected to be able to promote obesity. A key target of obesogens could be the peroxisome proliferator-activated receptor γ (PPARγ) because of its role in the induction of adipogenesis. Screening for PPARγ-active compounds has become a priority; the aim of this study was to establish the PPARg2-CALUX® assay in house at Nestlé to determine if it should be included in a battery of tests used for the *in vitro* screening of food-related materials including packaging. The PPARg2-CALUX® assay was successfully implemented in house; results obtained on a number of compounds demonstrated that it performed in agreement with the literature. However, application of an already-developed S9 metabolizing protocol on the PPARg2-CALUX® assay failed to induce changes in the response of tested compounds; *in silico* data suggested that phase I metabolism of a ligand is not expected to significantly influence its binding to the PPARγ active site. The CALUX assay was later applied to a set of compounds from the BPx family because of their suspected obesogen effects. Our results revealed antagonistic potential for all compounds (BPA, BPF, BPS, TCBPA, TBBPA, BADGE and BFDGE), but surprisingly only TCBPA and TBBPA were able to activate PPARγ-dependent transcription. Other modes of obesogenic action of chemicals, which are not detected by this assay, may be more important than previously suspected. Therefore, more understanding of the field is necessary before considering this assay as a first priority in routine screening *in vitro* test battery.

PS 2432 PPARα Activation Drives Zone-Specific Transcriptional Programs in the Rat Liver

P. D. McMullen, S. N. Pendse, B. Parks, E. L. LeCluyse, R. A. Clewell and M. E. Andersen. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

The liver is comprised of millions of parallel functional units (lobules) each containing several hundred hepatocytes. The physiology and metabolic specialization of hepatocytes differ greatly between these zones: periportal hepatocytes are smaller and primarily engage in oxidative energy metabolism, whereas centrilobular hepatocytes are large, polynucleated cells specializing in glycolysis and xenobiotic metabolism. Zone-specific expression of proteins in liver tissues has been previously observed upon chemical perturbation of other ligand-activated receptor systems (e.g., AhR). To better understand zonal biology and its relationship to chemical sensitivity, we used laser capture microdissection (LCM) to evaluate zonal differences in transcriptomic response to PPARα activation. Male Sprague-Dawley rats were treated with a selective agonist of PPARα (GW7647) at 0, 0.1, 0.25, 1.0, 2.5, 10, or 20 mg/kg/d via oral gavage for 4 days. Livers from these animals were evaluated by immunohistochemistry or LCM of periportal, midzonal, and centrilobular hepatocytes. We observed zone-specific activation of PPARα-regulated marker proteins in the periportal region of the liver using immunohistochemistry. Gene expression microarray analysis of these subpopulations revealed that more genes were differentially expressed in periportal cells than in centrilobular (2837 vs 432 at 20 mg/kg/d). Additionally, the magnitude of the transcriptional changes was larger in the periportal subpopulation and the dose at which cells responded was lower (AC50 = 0.38 vs 1.94 mg/kg/d). Analysis of canonical PPARα transcriptional targets, as well as pathway analysis using BMDExpress, revealed that all three zones respond with alterations in fatty acid metabolic machinery. However, there are a number of processes–including cell cycle, apoptosis, and cell-cell adhesion Gene Ontology categories–that are altered exclusively in periportal hepatocytes. Differential periportal responses may be a key step in tumorigenesis driven by PPARα agonists in rodent livers.

PS 2433 Use of Two Compounds to Validate Wild-Type (WT) and Constitutive Androstane Receptor (CAR) Knock-Out Rat Hepatocyte Cultures

A. Vardy[2], B. Elcombe[2], C. Elcombe[2], I. Fegert[1], M. Goettel[1] and N. Honarvar[1]. *[1]BASF SE, Ludwigshafen, Germany and [2]CXR Biosciences, Dundee, United Kingdom.*

Isolated rodent hepatocytes are a convenient *in vitro* stepping stone routinely used to model enzyme induction and hepatocyte proliferation detected during *in vivo* rodent studies. This study utilised 3-(difluoromethyl)-1-methyl-N-(3′,4′,6′-trifluoro[1,1′-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide with cytochrome P450 (CYP) 2B inducing potential (Test Item 1) and Fluquinconazole (FQZ; a triazole inducing liver tumours in mice and rats with known CAR-activating potential) to further validate isolated wild-type (WT) rat hepatocytes and to compare with those isolated from CAR knock-out (CAR KO) rats (SAGE Labs, Boyertown, PA) to determine the contribution of this nuclear hormone receptor to the mode of action. The two compounds were administered to hepatocytes for 96 hours at various concentrations up to cytotoxic levels as determined by ATP depletion and assessed for hepatocyte proliferation and enzyme induction. Test Item 1 and FQZ caused induction of CYP2B and CYP3A in WT rat hepatocytes as measured by increased pentoxyresorufin-O-depentylation (PROD), benzyloxyresorufin-O-debenzylation (BROD) and benzyloxyquinoline-O-debenzylation (BQ) activities, whereby at top concentrations competitive enzyme inhibition occurred. In the CAR KO rats, there was essentially no effect on PROD or BROD activities with either compound. However, BQ activity was similar to that measured in the WT hepatocytes. Both Test Item 1 and FQZ increased the hepatocellular labelling index, as a marker of S-phase induction, in WT hepatocytes. However, the increase in labelling index was absent in the CAR KO hepatocytes. Taken together these models have generated data demonstrating that a functional CAR is required for both Test Items to induce S-phase (replicative DNA synthesis) and CYP2B activity, given the lack of response in the CAR KO rat model. However, the CAR KO model demonstrates that both Test Items may also activate PXR, given the BQ activity induction observed in both WT and CAR KO hepatocytes.

PS 2434 Comparative Genomic Profiling of Constitutive Androstane Receptor with Direct and Indirect Activators in Humanized Mice

B. Niu[2], A. Bataille[2], F. Pugh[2], I. Albert[2] and C. J. Omiecinski[2]. *[1]Center for Molecular Toxicology and Carcinogenesis, Department of Veterinary and Biomedical Science, Pennsylvania State University, University Park, PA and [2]Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA.*

The constitutive androstane receptor (CAR) is a key nuclear receptor that transcriptionally regulates diverse biochemical and physiological processes, including bile acid and xenobiotic metabolism, as well as lipid and energy homeostasis. Many of these CAR-regulated functional activities appear conserved between rodent and humans. However, CAR activation in mice, by direct ligands or indirect activators such as phenobarbital (PB), is causally linked to the development of hepatocarcinogenesis, while these processes do not appear to occur in humans. These observations suggest species selective genomic and regulatory interactions for CAR. In this study we used a transgenic adenovirus delivery system to enable hepatocytes in CAR knockout mice to express either human CAR or mouse CAR conjugated with YFP tag, allowing their visualization and also chromatin immunoprecipitation (ChIP) of CAR. Both CAR direct and indirect activators were used in this study. Thousands of CAR genomic targets were identified, including regulatory regions of genes that encode processes related to all three phases of biotransformation and an expanded array of CAR gene targets related to energy and cholesterol metabolism and cell cycle regulation. Novel binding sites such as cell cycle related gene Meig1 and Cdc20 as well as sites close to previously reported CAR associated genes such as energy metabolism related gene Scd1 and Pck1, suggesting direct regulation of CAR to these genes. Comparative analysis between direct and indirect

activation targets and cross-species target analysis reveals a unique CAR regulatory network concerting xenobiotic metabolism, energy homeostasis and cell cycle regulation. These differential analyses will allow a more detailed understanding of CAR in its diverse role as a biological regulator across species.

2435 Metabolomic Approaches Reveal the Role of CAR in Energy Metabolism

F. Chen, T. Chen, D. M. Coslo, L. Zhang, A. D. Patterson and C. J. Omiecinski. *Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA.*

The constitutive androstane receptor (CAR) is a nuclear receptor that is well recognized for its important regulatory role in biotransformation and xenobiotic transport function. More recently elucidated is CAR's contribution as a regulator of energy metabolism and lipid homeostasis. In this study, we used metabolomic approaches to undertake comprehensive global analyses of metabolites and pathways linked to glucose, lipid, and fatty acid metabolism. TCPOBOP, a specific mouse CAR activator, was intraperitoneally administered to wild-type and CAR knockout mice. Subsequently, global serum and liver tissue metabolomes were analyzed using NMR, GC/MS and UPLC/MS-MS-based analytical methods. Results indicated that the levels of lactate, ketone bodies and tricarboxylic acid cycle products significantly increased, but the levels of phosphatidylcholine (PC), sphingomyelin and glucose levels were significantly decreased after 48h activation of mCAR in wild-type mice, but not in CAR knockout mice. Real-time PCR was performed to examine hepatic mRNA expression of several key rate-limiting enzymes involved in these energy metabolism pathways. Combined with the metabolomic data, activation of mouse CAR by TCPOBOP affects fatty acid synthesis and beta-oxidation, inhibits hepatic gluconeogenesis and cholesterol synthesis, and significantly reduces the level of PC, which was mainly contributed through accelerating the degradation of PC. In conclusion, the results of our metabolomics investigations now provide a view of the global profile of glucose, lipid and fatty acid metabolism pathways that are regulated by CAR and reveal potential therapeutic modalities for metabolic diseases that may be managed by CAR interventions.

2436 Characterisation of the Hepatic Effects of Phenobarbital (PB) and Pregnenolone 16A-Carbonitrile (PCN) in Constitutive Androstane Receptor (CAR, Nr1i3) and Pregnane X Receptor (PXR, Nr1i2) Double Knockout Rats

L. Chatham, C. Haines and C. Elcombe. *CXR Biosciences, Dundee, United Kingdom.*

The activation of the nuclear receptors CAR and PXR in rat liver results in hepatocellular hypertrophy and hyperplasia. This study investigated the effects of the CAR activator PB and the PXR activator PCN in CARKO/PXRKO rats (SAGE Labs, Boyertown, PA.). Male Wild Type (WT) and CARKO/PXRKO rats (n=5), were administered either PB in the diet (500 ppm) or PCN (100 mg/kg) p.o. daily for 7 days. WT and KO rats were administered control diet or corn oil p.o. as controls. All rats were implanted with osmotic pumps (7 day) containing BrdU, to allow determination of replicative DNA synthesis (S Phase). Treatment of WT rats with PB resulted in a 1.2-fold increase in liver/body weight ratio, a 7.7-fold induction in hepatocellular S-phase labelling index and 1.8-fold increase in total microsomal P450. Treatment of CARKO/PXRKO rats with PB did not increase liver/body weight ratio, hepatocellular S-phase labelling index or microsomal P450 content. Following PB administration, the microsomal CYP2B catalysed reactions pentoxyresorufin-O-depentylation (PROD) and benzyloxyresorufin-O-debenzylation (BROD) were induced 93- and 145-fold, respectively in WT rats. Induction of PROD or BROD was not observed in CARKO/PXRKO rats treated with PB. WT rats treated with PCN resulted in a 1.2-fold increase in liver/body weight ratio, a 13.8-fold induction of hepatocellular S-phase labelling index and a 1.5-fold increase in total microsomal P450. Treatment of CARKO/PXR KO rats with PCN did not affect any of these parameters. PCN induced the microsomal CYP3A catalysed reaction benzyloxyquinoline debenzylation (BQ) 4.8-fold in WT rats. BQ induction was not observed in CARKO/PXRKO rats after PCN treatment. Furthermore, CYP2B1, CYP2B2 and CYP3A1 mRNA levels were induced by either PB or PCN in WT but not CARKO/PXRKO rats. In conclusion, an active CAR is required for PB-mediated effects and an active PXR is required for PCN-mediated effects in rats.

2437 Pregnane X Receptor Plays a Role in Enhancing Resistance of Colon Cells to the Infection of *Salmonella typhimurium*

M. Wang[3], S. Ke[3], J. Wu[3], S. Lawhon[3], G. Wu[3] and Y. Tian[3]. *[1]Department of Animal Sciences, Texas A&M University, College Station, TX; [2]Veterinary Pathobiology, Texas A&M University, College Station, TX and [3]Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.*

Pregnane X receptor (PXR) is a nuclear receptor that ligand-dependently regulates gene expression of the xenobiotic metabolizing enzymes in the gastrointestinal (GI) tract. In recent years, PXR has been found to have novel functions in maintenance of homeostasis of the GI tract through interactions between commensal microbiota and host mediated by the metabolites from the commensal microflora. In this study, we found the PXR plays a role in regulating the innate resistance of the colon cancer cells to the infection of Salmonella typhimurium. In our infection assay, PXR-HCT116 and PXR-HT29 (colon cancer cell lines transfected with PXR) free of antibiotics were pre-treated with PXR ligands (24 h) and then inoculated with the Salmonella for 60 min. After the inoculation, the cells were washed 3 times with PBS and cultured in medium containing gentamicin. The intracellular bacteria were then released with the PBS containing 1% Triton X 100. The bacteria counts were enumerated through LB agar plating. Activation of PXR by rifampicin and hyperforin resulted in significant reduction of the intracellular bacteria. However, the PXR antagonist ketoconazole treatment reversed the inhibitory effects by PXR agonists. Interestingly, the PXR-imposed inhibitory effects on bacterial infection could be antagonized by co-treatment with lipopolysaccharides (LPS) which activates NF-κB pathway through Toll like receptor 4 (TLR4), suggesting the mutual repression between PXR and NF-κB also play a role in regulating the innate resistance to the Salmonella infection. Taken together, our results suggest PXR play a role in regulating GI tract resistance to bacterial infection.

2438 Alternative Exon 3 Inclusion in the Vitamin D Receptor (VDR) Modulates Transcriptional Activity for Target Genes: VDR, CYP24A1, CYP3A4 and CYP3A5

A. J. Annalora, P. L. Iversen and C. B. Marcus. *Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR.*

Xenobiotic exposures may induce alternative splicing in the pre-mRNA of nuclear hormone receptor (NHR) genes, yielding unpredictable patterns of gene expression that may promote adverse drug events and environmental disease. Informed by observations in the androgen receptor (AR; class I NHR), whereby alternatively-spliced AR transcripts induce ligand-independent hormone signaling via interactions with the wild-type (WT) AR, we designed an analogous system for probing similar phenomenon in the vitamin D receptor (VDR). The VDR is a prototype, class II NHR that regulates transcription of itself, and several cytochrome P450 (CYP) genes (e.g. CYP24A1, CYP3A4 and CYP3A5) linked to vitamin D metabolism. We hypothesized that deletion of exon 3, which encodes the DNA-binding domain (DBD) in all NHRs, would create a dominant negative VDR capable of modulating WT VDR function in a ligand-independent manner. To explore this concept, a synthetic, exon 3 deleted VDR (DEx3) construct, which remains in-frame, was prepared and stably-integrated into Caco-2 (TC7) cells expressing WT VDR. Baseline transcription levels for VDR, CYP24A1, CYP3A4 and CYP3A5 were measured by quantitative RT-PCR (qPCR), and compared to levels from cells stably-integrated with the DEx3 construct. In the absence of calcitriol, DEx3 derepresses VDR transcriptional activity, potentiating a > 4000-fold increase in CYP24A1 transcript levels. In the presence of calcitriol (500 nM), DEx3 VDR is a dominant negative at the promoters of the VDR and CYP3A4, but not CYP24A1 or CYP3A5. Furthermore, DEx3 VDR interacts differentially with the CYP3A4 promoter in the presence of calcitriol, unlike the CYP3A5 promoter, suggesting a more complex steroidogenic response, possibly linked to interactions with the pregnane X receptor (PXR). Because transcript variants of NHR genes are increasingly encountered via RNAseq methods, the discrete phenotypic changes associated with their expression warrant further attention.

2439 Identifying a Pharmacophore for the Estrogen Receptor Alpha/Beta Heterodimer to Decipher Its Biological Role

C. Coriano, C. Sievers and W. Xu. *Oncology, University of Wisconsin-Madison, Madison, WI.*

Estrogens regulate growth and development through the action of two main estrogen receptors (ERs): ERα and ERβ. They follow the classical nuclear receptor transcription factor mechanism of dimerizing upon ligand binding, translocating into the nucleus, and initiating gene transcription, thus regulating several processes related to cell proliferation and cell growth. Xenoestrogens can interfere with normal ER signaling which may lead to abnormal cellular proliferation and survival. The ligand binding domain of these receptors mediates dimerization where three dimer pairs are formed: ERα/α and ERβ/β homodimers and ERα/β heterodimers. They exhibit diverse physiological responses with ERα/α homodimers being pro-proliferative and ERβ/β homodimers being anti-proliferative with the biological role of the ERα/β heterodimer still unknown. Thus, the impacts of xenoestrogens on heterodimer activity are largely unknown. The purpose of this study is to identify a small molecule that promotes the formation and activation of ERα/β heterodimers to be able to decipher its biological role. A multistep small molecule screening study was done to identify a selective heterodimer-inducing compound that included: screening phytoestrogenic compounds for ER transcriptional activity using reporter assays and ER dimer selectivity using a Bioluminescent Resonance Energy Transfer Assay (BRET). Followed by an *in silico* study to identify the pharmacophore that confers ERα/β heterodimer specificity and 3D virtual screening of two commercial compound libraries. The cell effects of the compounds identified from this screening were then characterized using cell based assays. Here we report the discovery of 6 compounds that can selectively activate ERα/β heterodimers at specific concentrations and we demonstrate that the induction of ERα/β heterodimers are growth inhibitory in breast and prostate cells which co-express the two ER isoforms. The co-expression of ERα and ERβ in the same cells supports the possibility of ERα/β heterodimer formation at physio- and pathological conditions, further suggesting that targeting ERα/β heterodimers might be a novel therapeutic approach to the treatment of cancers which co-express ERα and ERβ.

2440 Evaluation of Thrombosis with an Accelerated Time to Occlusion (ATTO) *In Vivo* Swine Model

S. Gabriel, A. Sawyer and D. Nazarenko. *Corporate PreClinical Development and Toxicology, Becton Dickinson, Durham, NC.*

Currently, there is a need for an improved thrombogenicity detection method for the determination of biocompatibility for intravenous devices and materials, such as catheters. Present models are problematic in their evaluation of thrombogenicity and are performed with several caveats. This study was performed in order to establish a reproducible model for the detection and evaluation of thrombogenicity in catheters and catheter like devices. This model uses pressure changes to detect and determine occlusion (thrombosis generation) in catheters. Male fasted swine were anesthetized and fluids were administered throughout testing in order to normalize the blood pressure. The catheters tested were 22 gauge peripheral venous catheters (fluorinated ethylene propylene (FEP) 0.7x19mm catheter and a 0.9x25mm polyurethane (PE) catheter). Syringes were filled with saline for injection and placed on a Harvard pump. Pressure transducers were attached to the syringes with extension sets forming a continuous fluid path. As catheters were placed in the mammary veins of the swine, the extension set was attached and a small amount of fluid flushed through to ensure patency. An Aegis Pressure System was used to monitor and record pressure changes. A withdrawal and infusion program was used to withdraw a small amount of blood, hold this blood in the catheter for 3 minutes, then flush with injectable saline through the catheter and finally hold for 2.5 minutes before repeating the sequence. Repetition continued until the pressure tracings generated showed an increase above baseline pressure of at least 4psi, indicating an occlusion present in the catheter. A total of 37 FEP catheters and 38 PE catheters were tested. The average time to occlusion (TTO) for the FEP catheters was 37.63+/-11.83 min. The average TTO for the PE catheters was 74.04+/-23.36 min. It was concluded that the FEP and PE catheters of identical gauge are significantly different in their TTO under the same conditions. This conclusion indicates that this *in vivo* ATTO model in swine is sensitive enough to detect thrombogenic potential differences in two intravascular devices of different materials.

2441 Safety Assessment of Radiofrequency Renal Denervation in Swine

J. H. Keating[3], J. R. Stanley[3], A. R. Tzafriri[3], A.-M. Spognardi[3], P. M. Markham[3] and E. R. Edelman[2]. [1]*Applied Sciences, CBSET Inc, Lexington, MA;* [2]*IMES, MIT, Cambridge, MA and* [3]*Pathology, CBSET Inc, Lexington, MA.* Sponsor: S. Gad.

Radiofrequency (RF) renal denervation (RDN) is a novel catheter based treatment option for patients with refractory hypertension, wherein energy is delivered at the renal artery wall lumen by a single or multiple RF electrodes. Efficacious reductions in blood pressure (BP) are associated with inhibition of sympathetic signaling and require the delivery of sufficient RF energy to functionally transect efferent and afferent nerves. Variable clinical results have been correlated with shallow energy penetration, as demonstrated in postmortem histopathologic sections. Thus, RF ablation zones must be sufficiently deep and extensive to allow for effective nerve disruption, while minimizing adverse effects on treated vessels (e.g., thrombosis, excessive neointima formation or arterial wall injury) and on regional tissues such as adventitial soft tissue and vasculature, skeletal muscle, adrenal glands and ureters. We have used histopathology, immunohistochemistry, and morphometry to spatially map the presence of RF-induced changes in arterial and periarterial tissues after multielectrode RF catheter treatment. Correlation of nerve effects with biomarkers (e.g. reduction in renal norepinephrine) revealed a threshold dependence that could be predictably modulated by altering the number of RF treatments. Computational modeling of energy and heat transport correlated with histopathologic observations in the swine model. Variability in response to treatment resulted from differences in nerve distribution patterns and the presence of structures such lymph nodes and blood vessels that can draw RF power and dissipate heat.

2442 Safety Assessment of an Intramedullary Bone Stabilization Device Using a Light-Curable Monomer

B. G. Zani[2], R. Baird[2], J. R. Stanley[2], P. M. Markham[2], M. Wilke[1], S. Zeiter[1], A. Beck[1], D. Nehrbass[1], G. A. Kopia[4], E. R. Edelman[5] and R. Rabiner[3]. [1]*AO Foundation, Davos, Switzerland;* [2]*CBSET, Inc., Lexington, MA;* [3]*IlluminOss Medical, Inc., East Providence, RI;* [4]*Kopia Consulting, Hillsborough, NJ and* [5]*IMES, MIT, Cambridge, MA.* Sponsor: S. Gad.

Traditional methods of bone fixation (e.g., metal rods and screws) are not effective for osteoporotic patients. A device for percutaneous bone stabilization utilizing a light-curable polymer system that conforms to each patient's unique intramedullary (IM) canal has been developed. We evaluated local and systemic biocompatibility of a photodynamic balloon stabilization system (PBSS) cured *in situ* in sheep. Tibiae in 19 sheep were implanted with a single PBSS and harvested at 30, 90 or 180 days. Another 36 sheep received a mid-shaft tibial osteotomy stabilized with either external fixators or external fixators combined with an IM PBSS and were evaluated at 8, 12 and 26 weeks. Healing and safety were assessed by radiographic analysis, micro computed tomography and/or histopathology. Non-fractured tibiae showed no significant macroscopic or microscopic observations. Implants conformably filled the IM space. Active cortical bone remodeling and apposition of new periosteal and/or endosteal bone were observed. Osteotomized tibiae displayed continuous bone formation inside the osteotomy gap with no significant toxic effects when fixation was augmented by the PBSS. Periosteal callus size decreased over time and was similar in both groups. Foamy macrophages were observed locally at the implantation site in some PBSS-treated animals and clusters of foamy macrophages were observed in local lymph nodes both in controls and PBSS-treated animals. No inhibition of endosteal bone remodeling and/or vascularization was observed. Macroscopic observations at necropsy exhibited no pathological changes in the spleens of treated animals. IM application of a light curable PBSS is a safe, feasible method for IM fixation.

2443 Effect of Components in Resin-Based Dental Restoratives on Protein-S-Glutathionylation

J. T. Samuelsen, S. Uvsløkk, E. Morisbak, R. Becher and J. E. Dahl. *NIOM, Oslo, Norway.*

Protein S-glutathionylation (PSSG) is the reversible formation of mixed disulfides between glutathione and protein cysteinyl residues. This process is suggested to be a physiologically central redox signaling mechanism. Several methacrylates that are known to leak from resin based biomaterials, form adducts spontaneously with the cysteine-thiol of glutathione (GSH) and causes increased cellular levels of reactive oxygen species (ROS) *in vitro*. Both these changes could potentially influence

PSSG. Increased oxidative stress may shift the state of protein thiols against an oxidized state (PSSG), while a reduced level of PSSG could be a result of GSH-depletion. In this study we aim to examine if methacrylate exposure affect the cellular PSSG state. The human bronchial epithelial cell line BEAS-2B grown in Lechner and LaVeck (LHC9) medium was used as a model system. The cells were exposed to methylmethacrylate (MMA), 2-hydroxyethylmethacrylate (HEMA), triethyleneglycol-dimethacrylate (TEGDMA) and glycerol-dimethacrylate (GDMA). The MTT assay was used to determine cell viability after 24 h exposure. Cellular GSH and ROS levels were measured by flow cytometry after 4 h exposure using the fluorescent probes monobromobimane (MBBr) and dichloro-dihydro-fluorescein diacetate (DCFH-DA), respectively. After 4 h exposure, the cellular PSSG state was visualized by western blotting using a PSSG specific antibody. No change in viability was detected by the MTT assay after exposure to <16 mM MMA, <8 mM HEMA, <2 mM TEGDMA and <1 mM GDMA. Using these concentrations, significant glutathione depletion and increased cellular ROS were measured in cells exposed to HEMA, TEGDMA and GDMA. No changes were observed in MMA exposed cells. Western blotting showed one prominent protein-band at approximately 40 kDa. Incubating the filter with antibodies to both β-actin and PSSG showed identical migration in the gel of the detected proteins. The intensity of the PSSG band decreased after exposure to all methacrylates, although not significantly in MMA exposed cells. In summary, this study shows that protein-S-glutathionylation of at least on protein, probably β-actin, is reduced in BEAS-2B cells after exposure to methacrylates. The decrease was most evident after exposure to methacrylates with higher thiol-reactivity (higher GSH-depletion observed).

PS 2444 Exposure to Dental Monomer Hydroxyethyl Methacrylate (HEMA) Induces Increased Transcription of Genes Involved in Oxidative Stress Responses

R. Becher[1,2], H. V. Rukke[1], J. E. Dahl[1] and J. T. Samuelsen[1]. [1]*Nordic Institute of Dental Materials, Oslo, Norway and* [2]*Norwegian Institute of Public Health, Oslo, Norway.*

Polymer-based restorative materials are widely used in dentistry. Cured materials contain unpolymerized methacrylate monomers which leak into the oral environment. Several studies have described adverse biological effects including apoptosis *in vitro* after short term exposure to relatively high concentrations of the leachable hydroxyethyl methacrylate (HEMA). The mechanisms involved in the cytotoxic effects of HEMA have not been fully elucidated although involvement of various signaling pathways including oxidative damage, activation of MAP-kinases as well as caspases has been suggested. Involvement of reactive oxygen species (ROS) formation is supported by our previous finding that HEMA influence intracellular levels of glutathione which play a dominant role in protection against oxidative damage. To further elucidate mechanisms involved in toxic responses induced by HEMA, we exposed the cell line BEAS 2B to a "sub lethal" concentration of 2 mM HEMA for 4 hours and subsequently isolated mRNA for analysis of the mRNA expression profile (transcriptomics). The results showed that HEMA clearly, but not only, induced increased mRNA levels of Ovary, Kidney and Liver Protein 38 (OKL38), Heme Oxygenase 1 (HMOX1), Thioredoxin Reductase1 (TXRND1) and Glutamate-Cysteine Ligase, Modifier subunit (GCLM). We have previously shown that HEMA induces expression of tumor suppressor protein p53. OKL38 has been identified as a downstream target of p53 with a possible role in regulating cell death in concordance with p53. OKL38 has also been shown to be an oxidative stress response gene stimulated by oxidized phospholipids. Furthermore, HMOX1 and TXNRD1 encode proteins associated with protection against oxidative stress whereas GCLC encodes the first rate limiting enzyme of glutathione synthesis. Overall, our results support that HEMA causes oxidative damage followed by activation of genes involved in protection against oxidative stress in the BEAS 2B cell line.

PS 2445 Proteome Changes Indicate Oxidative Stress and Protein Damage after Methacrylate Exposure in Human Monocyte Cell Line

J. E. Dahl[1,5], V. Barman Michelsen[3], J.-A. Bruun[2], E. Jensen[2], U. Örtengren[2] and J. T. Samuelsen[1]. [1]*Nordic Institute of Dental Materials, Oslo, Norway;* [2]*Department of Clinical Dentistry/ Faculty of Health Sciences/, University of Tromso, Tromso, Norway;* [3]*Department of Clinical Dentistry/Faculty of Medicine, University of Bergen, Bergen, Norway;* [4]*Department of Pharmacy/Faculty of Health Sciences, University of Tromso, Tromso, Norway and* [5]*Institute of Clinical Dentistry, University of Oslo, Oslo, Norway.*

Scope: To study the initial changes in the proteome in a human monocyte cell line after exposure to methacrylate monomer, The study was performed using the SILAC (Stable Isotope Labelling with Amino Acids in Cell culture) method. Human THP-1 monocyte cells (ECACC, the European Tissue Type Culture Collection) were cultured in either heavy medium (medium containing 13C labeled L-Arginine and 13C labelled L-Lysine) or light medium containing same concentration of unlabeled (12C) L-Arginine and L-Lysine. After eight cell divisions, cells cultured in heavy medium were exposed to the methacrylate monomer 2-hydroxymethacrylate (HEMA; CAS. 868-77-9, Fluka Chemie AG, Buchs, Switzerland) for 24 h and mixed with the same amount of control cells cultured in light medium. To correct for experimental errors, a label-swap replication was performed (exposed cells in light medium mixed with control cells in heavy medium). The ratio of cellular proteins containing heavy and light arginine and lysine were determined by shotgun proteomic analyses. Tryptic peptides of the samples were analyzed with LC/MS-MS (liquid chromatography/double mass spectrometry). Proteins were identified and quantified using Max Quant and Proteome Discoverer software. Cell viability was assessed by the MTT assay. Results: The MTT assay did not reveal any effects of HEMA at the concentrations used. Increased levels of several proteins indicative of adaptation to oxidative stress (e.g. pirin, glutathione reductase and thioredoxin reductase 1) were measured in the HEMA exposed cells. Increased levels of Hsp70 family proteins indicate protein damage after HEMA exposure, possibly as a result of increased oxidative stress. The effects increased with increasing HEMA concentration. Conclusion: It has been suggested that reactive oxygen species are involved in HEMA toxicity. The upregulated levels of antioxidant enzymes and Hsp70 family proteins showed that HEMA exposure lead to cellular stress at concentrations that had no effect on cell viability.

PS 2446 Comparison of Cytotoxicity Results from Two Testing Laboratories Using ISO Mem Elution and Colony Formation Method

Y. Zhou[4], M. Posgai[4], D. Malek[1], J. Kringstad[3], A. Zdawczyk[2] and R. T. Przygoda[4]. [1]*Malek Toxicology Delaware LLC, Greenville, DE;* [2]*NAMSA, Northwood, OH;* [3]*Wuxi AppTec, Inc, St. Paul, MN and* [4]*Life Cycle Materials, Johnson & Johnson, Cincinnati, OH.*

Two cytotoxicity tests (the MEM elution (ME) and Colony Formation (CF)) are commonly used in the biological evaluation of medical devices. In this study, five commonly used positive control materials (powder-free latex (PFL), black rubber (BR), cadmium chloride, ZDBC and ZDEC), either steam-sterilized or non-sterilized, were tested for cytotoxicity at two testing laboratories under GLP to investigate 1) inter-laboratory variations; 2) the impact of the sterilization on the cytotoxicity, and 3) the consistency of the ME and CF results. A serial dilution of the test sample extract was tested for ME (ranging from 0.39% to 100%) and CF (ranging from 0.098% to 100%) according to ISO 10993-5 standard. Inconsistent results were observed not only between the testing laboratories, but also between the ME and CF testing method for the non-sterilized BR (Lab A: non-toxic (ME, scores 2 at 100%), toxic (CF, IC50=0.95%); Lab B: toxic (ME, scores 4 at 100%), non-toxic (CF)). When steam-sterilized, the BR did show cytotoxicity in ME and CF in both labs. An unusual response for the unsterilized PFL in ME (i.e. scores of 2 (non-toxic) for doses 3.25% to 100% except for 6.25%, which scores 4 (toxic)) was reproducible in one lab, but not the other. Consistent results were shown for ZDEC, ZDBC, cadmium chloride, steam-sterilized BR, and steam-sterilized PFL among labs and testing methods. The comparisons suggest that the test article preparation may contribute to the variability between the testing labs, especially for materials with equivocal results. The sterilization could impact the cytotoxicity; and ME and CF tests provide comparable results (83% agreement in the current study).

2447 Resolving Hemolysis Test Failures in the Biological Evaluation of Blood-Contacting Medical Devices

F. K. Hsia[1], N. S. Goud[1], A. Andrews[3] and H. A. Schwanz[3]. [1]*Global Toxicology and Biocompatibility, Pre-Clinical Sciences, Boston Scientific Corporation, Marlborough, MA;* [2]*Pharmaceutical Sciences, Interventional Cardiology Research and Development, Boston Scientific Corporation, Maple Grove, MN and* [3]*Quality Assurance, Boston Scientific Corporation, Maple Grove, MN.*

All blood contacting devices (either externally communicating or implantable) are required to be evaluated for hemolysis test as per ISO 10993-1 Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing within a risk management process. The FDA draft guidance (2013) on ISO 10993-1 recommends both direct and indirect (extract) methods to be conducted per ASTM F756 or an equivalent method. Two case studies on hemolysis are presented demonstrating false positive results in the ASTM method. In the first case, the implantable device consisted of bioabsorable polymer and an active pharmaceutical ingredient. While hemolysis by the direct contact method passed the acceptance criteria (Hemolytic Index HI < 5.0), the indirect method has failed (HI = 7.2) when the device was extracted per ISO 10993-12 recommended conditions of 50°C for 72 hours at 0.2 g/ml. Later investigations showed that the test failure could be due to accelerated degradation of the polymer (acidic pH < 2.4) at aggressive extraction conditions. Repeat testing using clinically relevant extraction ratio (0.075 g/ml) and at 37 °C for 72 hours showed passing result (HI = 2.5, pH = 4.4). In the second case, a liquid of intralipid emulsion (consisting of lipids, oil and glycerin) used as lubricant in an atherectomy device was the test article. This test article did not meet the ASTM direct hemolysis acceptance criteria. Investigation revealed that the absorbance values were outside the range of the standard curve due to turbidity of test article. Therefore, the assay was repeated by diluting the test article in saline as per instructions in the DFU and corrected for background absorbance. The repeat assay showed no hemolysis indicating the need to adjust test conditions depending on the clinical use of the device. In summary, the above two examples demonstrate that hemolysis test failures could be due to artifacts and limitations of *in vitro* test systems. Therefore, due care should be taken in interpreting any false positive data and consider performing the tests simulating the clinical use of the device.

2448 Are the Current Medical Device Extraction Procedures Sufficient for Genetic Toxicity Hazard Identification?

R. T. Przygoda[2], M. Posgai[2], Y. Zhou[2] and D. Malek[1]. [1]*Malek Toxicology, Greenville, DE and* [2]*Life Cycle Materials, Johnson & Johnson, Cincinnati, OH.*

Extracts prepared according to ISO 10993-12 are used in several genetic toxicity test systems to identify potential hazards. The adequacy of the various extraction procedures allowed in ISO 10993-3 is still being debated. The concern is that these extraction procedures may not be adequate for hazard identification. The literature was searched for the lowest positive dose (LPD) induced by various mutagenic chemicals in each of the following tests: bacterial reverse mutation, *in vitro* chromosomal aberration (CA), and the *in vivo* micronucleus. For the bacterial reverse mutation test and the *in vivo* micronucleus test, results from 161 IARC carcinogens were evaluated. For the *in vitro* chromosomal aberration test, results from 474 clastogenic chemicals were extracted from a report by M. Ishidate, et. al. (1988). The cumulative frequency (CF) of LPDs at selected extract concentrations was determined and used to assess the percentage of mutagens detected at each these concentrations. This comparison was used to determine if the extract concentrations were sufficient to induce a mutagenic response at the LPD. In addition, results from 104 Non-Volatile-Residue (NVR) tests were analyzed to determine the CF of NVR at 6 targeted dose-concentrations (0.001% to 10%). When device extract concentrations were ≤ 0.1%, which occurred 83% of the time in this analysis, the percentage of mutagenic chemicals that could be detected was less than 50% with the exception of the CA test using media. The CA test using media could detect 76% of the mutagens at 0.1%. Therefore, for extract concentrations of 0.1% or less, the ISO 10993-12 method was considered inadequate for genetic toxicity hazard identification. When device extract concentrations were > 0.1%, which occurred 17% of the time in this analysis, the percentage of mutagenic chemicals that could be detected varied between 36% and 100%. At 1% concentration, 36% to 76% of the mutagens could be detected. At 10% concentration, between 76% and 100% of the mutagens could be detected. Therefore, the ISO 10993-12 extraction method was considered inadequate at 1% and acceptable at 10%.

2449 *In Vitro* Hemocompatibility Evaluation of Nanocrystalline Diamond Coatings

S. A. Skoog[1,2], Q. Lu[2], R. A. Malinauskas[2], A. V. Sumant[3], J. Zheng[2], P. L. Goering[2], R. J. Narayan[1] and B. J. Casey[2]. [1]*UNC/NC State, Raleigh, NC;* [2]*US FDA, Silver Spring, MD and* [3]*Center for Nanoscale Materials, Argonne National lab, Argonne, IL.*

Nanocrystalline diamond (NCD) is an emerging biomaterial that exhibits mechanical robustness, chemical inertness, and biocompatibility. Due to these material properties, NCD thin films have been investigated for improved wear resistance and enhanced hemocompatibility of cardiovascular devices, such as artificial heart valves, stents, and ventricular assist devices. The goal of this study was to evaluate the *in vitro* hemocompatibility of NCD coatings in contact with human blood and determine the effect of nanoscale surface topography on blood-material interactions. NCD coatings were deposited on silicon substrates using microwave plasma enhanced chemical vapor deposition. By varying the hydrogen/argon gas ratio and deposition time, NCD coatings with small grain sizes (<100 nm) and coatings with large grain sizes (up to 400 nm) were synthesized in a reproducible manner. Physico-chemical characterization of the NCD surfaces was conducted using SEM, atomic force microscopy, contact angle testing, and Raman spectroscopy. *In vitro* hemocompatibility testing included evaluation of blood protein adsorption, hemolysis, non-activated partial thromboplastin time, platelet adhesion/aggregation, and activation of platelets on NCD surfaces compared to positive and negative control materials. NCD coatings demonstrated low protein adsorption, a non-hemolytic response, and minimal activation of the plasma coagulation cascade. Furthermore, NCD coatings exhibited low thrombogenicity with minimal platelet adhesion and aggregation, and morphological changes to surface-bound platelets (e.g., activation) in comparison to the HDPE negative control material. No statistically significant differences were observed between the NCD coatings with different nanoscale surface topographies for all tests. The *in vitro* data presented here suggests that the two tested NCD coatings, regardless of nanostructured topography, had similar hemocompatibility profiles compared to the negative control material (HDPE) and should be further evaluated for use in blood-contacting medical devices.

2450 *In Vitro* Biological Evaluation of Additive Manufactured Metal Discs

G. Kumar[3], J. Chang[2], R. Patel[1], J. Coburn[3], M. A. Di Prima[3], A. Nguyen[3] and P. L. Goering[3]. [1]*UMBC, Baltimore, MD;* [2]*University of Maryland, College Park, MD and* [3]*US FDA, Silver Spring, MD.*

Additive manufacturing (AM) is an emerging technology that offers personalized medicine applications for orthopedics, dental, and neurological medical devices. Some advantages of AM over conventional manufacturing are: it reduces the prototyping cost, it is easier to control design, it eliminates waste and design changes are easier to implement. The aim of this study was to determine cytotoxic and other cellular responses of 3D printed metal discs. Metal discs were 3D-printed using powder bed laser sintering. Ti-6Al-4V (Ti64) and cobalt chromium (CoCr) discs were printed in 2 orientations, with the flat side parallel to the print bed and vertical to it. Certain samples were then post-processed by sandblasting and light sanding. Cytotoxicity was evaluated by using both direct contact and indirect contact assays. For direct contact assay, human bone marrow stromal cells (hBMSCs) were seeded directly on the surface of metal discs and morphology evaluated by either fluorescence microscopy or SEM image analysis. For indirect contact assay, UV-sterilized discs of various 3D-printed metals (Ti64, 3D-Ti64-V, 3D-Ti64-H, 3D-Ti64-PP, 3D-Ti64-NPP, CoCr, 3D-CoCr-V and 3D-CoCr-H) were extracted per ISO 10993-12 guidelines (37oC x 24 hours in cell media; SA:V ratio of 3 cm2/ml) supplemented with 7.5% FBS. hBMSCs were grown in growth media for 24 hrs. Media was then replaced with extracts and cells were grown an additional 24 hr. Cytotoxicity was assessed quantitatively by MTT assay and qualitatively by using Live-Dead and mitochondrial assay. Direct contact assay results suggest that hBMSCs attached on all 3D printed metal discs. Extracts of 3D-Ti64-PP showed severe cytotoxicity for Live/Dead, mitochondrial and MTT assay (> 75% cell death) indicating that post processing can potentially alter the cytotoxicity of 3D-printed discs. The data indicate that print orientation does not affect the cytotoxicity of 3D-printed metal discs.

2451 Real Time Laser 3D Printing of Living Cells in Hydrogels with Two Photon Polymerization by Minimizing Chemical, Free Radical, and Phototoxicity

P. E. Petrochenko[1], P. Gruber[3], M. Markovic[3], R. Liska[3], J. Stampfl[3], P. L. Goering[1], R. J. Narayan[4] and A. Ovsianikov[3]. *[1]US FDA, Silver Spring, MD; [2]Inst of Applied Synthetic Chemistry, Vienna Univ of Technology, Vienna, Austria; [3]Institute of Materials Science and Technology, Vienna Univ of Technology, Vienna, Austria and [4]Joint Department of Biomedical Engr, UNC-Chapel Hill and NC State University, Raleigh, NC.*

3D printing using two-photon polymerization (2PP) has gained increasing popularity in the field of tissue engineering due to its submicron resolution and an increasing choice of photopolymerizable materials. The possibility of encapsulating live cells into 3D structures directly has been limited by considerable cytotoxicity from the radical photopolymerization during the printing process. In this study, we selected a least toxic photoinitiator, Eosin Y by screening cell viability across 3 cell lines with the Presto Blue assay. Free radical scavengers: vitamins C, E, and glutathione were further tested for their ability to preserve cell viability during the printing process. Impact of phototoxicity was mitigated by carefully selecting the parameters of the 3D printing process, such as laser power and scanning speed, based on the preliminary tests using a confluent layer of MG63 fibroblasts and Live/Dead stain. Successful encapsulation of MG63 osteosarcoma cells within a primitive 3D construct was demonstrated using the Eosin Y photoinitiator in conjunction with vitamin C in GelMOD,a methacrylated gelatin hydrogel. A second approach to preserving cell viability was shown by printing in 50% fetal calf serum with fibronectin to significantly prolong long term human vascular endothelial cell (HUVEC) survival of at least 72 hrs. The material, a novel hyaluronic acid vinyl ester matrix was shown to print complex durable and flexible high resolution cell constructs. With proper controls for cytotoxicity, 2PP 3D printing was shown to be a highly capable technique for fabricating custom scaffolds from a computer model for *in situ* cell encapsulation.

2452 *In Vitro* Thrombogenicity Assessment: Comparison of an Ovine *In Vivo* Venous Implant Model to a Circulating Blood-Loop Thrombogenicity Assay for Medical Devices

K. Grove, S. Deline and M. E. Smith. *American Preclinical Services, Minneapolis, MN.*

ISO 10993-4 thrombogenicity testing is widely used for meeting regulatory requirements for approval of blood-contacting medical devices. Although *in vivo* models are often part of testing and submission packages, there is a need for faster and less costly *in vitro* assays. This study describes the continuing validation of an *in vitro* blood-loop assay which integrates freshly harvested, minimally heparinized pumped ovine blood. 500 ml blood was collected from 7 healthy sheep and heparinized (1 IU/ml). Blood was introduced into four concurrently flowing loops containing 125 ml each. Either positive or negative control catheters or a commercially approved catheter was deployed into the blood flow in each loop. Routinely, up to four loops were prepared simultaneously. After 4 hours, the loop sections containing the test materials were opened, photographed *in situ* and evaluated for the presence of thrombus in accordance with standard *in vivo* thrombogenicity scoring for acutely implanted devices. After a minimum 4 week recovery period from the blood draw, each of the 7 sheep were used for an acute non-anticoagulated venous implant (NAVI) test using both jugular veins and both external femoral veins as implant sites. Two predicate devices along with one positive control and one negative control catheters were deployed in each animal uniformly distributed across the 4 implant sites in all 7 sheep. The animals were maintained in supine position for 4 hours after which they were given an IV bolus of heparin to eliminate post-mortem thrombus formation and then euthanized. The implanted veins were exposed, removed intact and the devices and implant sites were scored for thrombus formation. Devices in both the *in vitro* and the *in vivo* models were evaluated for thrombus formation using a scoring system based on a scale of 0-5, where: 0 = "no thrombus visible" and 5 = "severe, with thrombus covering >75% of material area". Median thrombogenicity scores over all 7 animals in both the *in vivo* and the *in vitro* assays were identical for the three different test articles: the positive controls scored a median value of 5, the negative controls scored a median of 0 and the test catheter scored a median of 1. The comparison between the *in vivo* and *in vitro* results support the premise that this *in vitro* blood loop model may potentially supplant the NAVI model for hemocompatibility testing.

2453 Cytotoxicity Evaluation of Additively Manufactured Polymeric Discs

L. Ricles[2], E. Gonzalez[2], V. Hitchins[2], M. A. Di Prima[2], J. Coburn[2], S. Oh[2], P. L. Goering[2] and G. Kumar[2]. *[1]CBER, US FDA, Silver Spring, MD and [2]CDRH, US FDA, Silver Spring, MD.*

Additive manufacturing (AM), often referred to as "3D printing", involves layer-by-layer deposition of materials with precise spatial control and resolution. While AM has traditionally been used for industrial (e.g. aerospace, automotive) applications, AM is driving innovation to development of personalized medical devices and biologic products containing living cells. The aim of this study was to evaluate the cytotoxicity and of additively manufactured samples. Cube or honeycomb structures were printed using [spell out name] (ABS; fused deposition modeling) or polymethylmethacrylate (PMMA; stereolithography) and samples were placed in sterile tubes. PMMA samples were briefly washed in isopropanol (10 min), followed by UV treatment (10 min), prior to storage. Physical characterization and print quality of samples was performed by using scanning electron microscopy and profilometry. Cytotoxicity testing was conducted per ISO 10993-5 using L-929 fibroblasts. Printed samples were extracted using ISO 10993-12 (37°C for 24 hours in cell culture media (10% FBS) using surface area: volume ratio of 3 cm2/mL). L929 cells were plated at a density of 1x104 cells/well in a 96 well plate and cultured for 24 hrs. The media was then replaced with the extracts and cells were cultured for 24 hrs. The cytotoxicity of the printed samples was assessed quantitatively by MTT and qualitatively using Live/Dead and mitochondrial staining. The results of the cytotoxicity studies indicate that printed ABS samples were not cytotoxic, but printed PMMA samples were highly cytotoxic (> 90% cell death). Further, print direction and print geometry do not affect cytotoxicity of additively manufactured polymer discs. These results showed that cytotoxicity of additively manufactured polymers is dependent on the printing method.

2454 Local Reactions to Subcutaneous Injection of HFAs

O. P. Green[2], A. Donnelly[1] and N. Lourens[3]. *[1]Bespak Europe, Cambridge, United Kingdom; [2]WCA-Consulting, Faringdon, United Kingdom and [3]WIL Research Europe, s-Hertogenbosch, Netherlands.*

The toxicology of hydrofluoroalkanes (HFAs) via inhalation exposure are well documented as a consequence of their widespread use as propellants in metered dose inhalers (MDIs), but toxicity from accidental exposure following use as a propellant for subcutaneous/intramuscular autoinjectors has not been evaluated. As the inhalation data only provides data on systemic toxicity and local effects on nasal mucosa from HFA exposure, a study was conducted to assess the possible local effects of accidental subcutaneous exposures to two different HFA liquid gas autoinjector propellants (227 [R-227 alone] and 410a [R-125 and R-32]). The test species was the minipig because of structural and physiological similarities of the dermal layers compared to that in humans. Since the extent of the potential local reaction was unknown, the minipigs were injected under a light general anaesthesia and allowed to recover slowly after the injections. Each minipig acted as its own control and two propellants were studied. The injection sites were monitored closely in a detailed observation regimen. Blood samples were taken at intervals to examine for indicators of inflammation or immunological reactions. After 7 days the animals were killed and histopathological examination of the skin layers at the injection sites was performed. In-life reaction consisted of an immediate localised swelling in all animals following the injection as a result of subcutaneous gaseous expansion of the liquid propellant in each case. This reaction completely resolved within 24 hours and there were no other clinical events observed. Whilst there appeared to be no difference between the two propellants used, the pathological examination of the tissues revealed a difference in the severity of the local reaction between the two propellants. It is hypothesised that this difference in severity of the local reaction could be related to a difference in physicochemical characteristics of the propellants or a difference in the local irritancy potential of the propellants.

2455 Dose-Dependent Effects on Rat Liver miRNAs 200a/b and 429: Potential Early Biomarkers of Liver Carcinogenesis

S. Plummer[1], P. Brown[2], M. Beltran[1], M. Millar[2], J. Wright[1] and R. Currie[3]. *[1]MicroMatrices Ltd, Dundee, United Kingdom; [2]MRC Centre for Reproductive Health, Edinburgh, United Kingdom and [3]Syngenta Ltd, Bracknell, United Kingdom.*

Three rat microRNAs (miRs) (Rn miR200a, 200b and 429) that are stably induced for up to 90 days by liver non-genotoxic carcinogens but not by non-hepatocarcinogens were identified (Koufaris et al., 2012) and these microRNAs may serve as early predictors (biomarkers) of heptocarcinogenic potential. To test this hypothesis in a proof of principle study we used RT-PCR to measure the dose-response of the levels of these miRs in the livers of rats treated with two hepatocarcinogenic and one non-hepatocarcinogenic pyrazole carboxamide succinate dehydrogenase inhibitors (SDHI1, SDHI2 and SDHI3, respectively). RNA was extracted from wistar rat liver FFPE samples (males and females) from 3 different 90 day repeat dose toxicity studies with these compounds at 3 dose levels. The mirRs were quantified by RT-PCR at the low, mid and high doses relative to control. SDHI1 treatment caused a dose-dependent increase in miR 200a, 220b and 429 in males and females. SDHI2 treatment also caused a significant ($p<0.05$) increase in miR200b in males and females at the high dose compared to control and there was a dose-dependent increase in miR429 in SDHI2 treated males (not stat sig). SDHI3 treatment did not cause any dose related changes in miR 200a, 200b and 429 relative to control. Our results suggest that assessment of miR 200b/429 levels has potential as a biomarker of the perturbation of pathways involved in hepatocarcinogenesis in wistar rats. Further work will be required to establish whether or not these miRs may serve as early biomarkers of hepatocarcinogenic potential in other compound classes.

2456 Toxicity Profiling of Flame Retardants in Zebrafish Embryos Using a Battery of Assays

A. Alzualde[1], A. Alday[1], M. Behl[2], R. R. Tice[2], R. R. Paules[2], A. Muriana[1] and C. Quevedo[1]. *[1]BBD-BioPhenix, San Sebastián, Spain and [2]NIEHS-NIH, Research Triangle Park, NC.*

The developing zebrafish embryo is increasingly used as an alternative animal model for evaluating toxicities. The aim of this study was to assess the toxicity of 10 flame retardants (FRs) of environmental concern using a battery of zebrafish toxicity assays. With the phasing out of brominated FRs (BFR), organophosphate FRs (OPFRs) are increasingly being used. However, there is limited information on their potential for inducing adverse health effects. In this blinded study, 2 BFRs and 8 OPFRs (6 non-halogenated, 2 halogenated) were tested for developmental toxicity, cardiotoxicity, behavioral alterations, and hepatotoxicity. A bioavailability study was conducted to measure the internal compound exposure by LC/MS/MS. To determine the risk of teratogenicity of the tested compounds, the presence of morphological and functional alterations were analyzed and a teratogenic index (TI) established as the ratio of the LC50/EC50. High TI values were obtained for 3 compounds - isopropylated triphenyl phosphate (IPP), tert-butylphenyl diphenyl phosphate (BPDP), and tricresyl phosphate (TCP) (EC50 ~2-5 µM), - that highlight the possible teratogenic potential of these compounds. In terms of cardiotoxicity, 4 of the 6 non-halogenated OPFRs tested (IPP at 100 µM, BPDP from 10 µM, TCP from 30 µM, and triphenyl phosphate from 10 µM) induced bradycardia followed by auricular failure, a specific and uncommon zebrafish cardiotoxic effect. 2,2′4,4′-tetrabromodiphenyl ether (BDE-47) specifically affected locomotor activity from 1 µM, concentration at which not other deficits were observed. Finally, hepatotoxicity was found after the treatment with 2-ethylhexyl diphenyl phosphate (EHDP) and BPDP, for both at 10 µM. In conclusion, the OPFR results support the need for more extensive testing and demonstrate the applicability of this battery of zebrafish assays. This abstract does not necessarily reflect the views of NTP/NIEHS.

2457 A Randomized, Double-Blind, Three Cohort Crossover Study Assessing Gastrointestinal Tolerability of Magnesium Sources in Healthy Adults

S. Ashmead[1], D. Liska[2], K. Sanoshy[2], C. Kern[2], K. M. Kelley[2] and J. Hartle[1]. *[1]Albion, Clearfield, UT and [2]Biofortis Innovation Services, Addison, IL*. Sponsor: J. C. Griffiths.

A clinical study was conducted to assess the GI tolerability and bowel habits of three different magnesium (Mg) mineral sources [MgB: magnesium bisglycinate; DMM: dimagnesium malate; MgC: magnesium citrate] at differing amounts in healthy adults. The study utilized a double-blind, controlled crossover three cohort design, in which 300, 450, or 600mg/d of a specific magnesium salt was compared against placebo in each cohort. Test periods were 4 days and washout between test periods was at least 3 days. Sixty-six subjects were randomized (N=22/cohort) and 65 completed the study. The number of bowel movements per test period was not significantly different, however, significant improvements in fecal consistency were seen for MgB and DMM compared to placebo. Specifically, the fecal consistency score for placebo was 3.89 ± 0.26, which increased to 4.52 ± 0.24 ($p<0.05$) for 600 mg MgB. Likewise, the fecal consistency score for placebo in the DMM cohort was 3.11 ± 0.22, and increased to 3.74 ± 0.24 ($p<0.05$) and 3.94 ± 0.25 ($p<0.01$) for 450 and 600 mg DMM, respectively. Although no significant differences were noted for the ease of passage ratings, the average scores supported the findings for fecal consistency, with scores for the 450 and 600 mg servings of both MgB and DMM suggesting improvement (e.g., lower scores indicating easier stool passage). No significant differences in bowel habits were found for the MgC cohort. GI symptoms, when noted, were generally mild with no significant differences across test products and placebo . Of the GI symptoms reported, flatulence was the most prevalent during both placebo and active study product consumption. No AEs related to Mg study products were reported during the trial . Overall, all study products (MgC, MgB, and DMM) were well tolerated, with significant improvements in bowel habits noted for DMM and MgB over placebo or MgC.

2458 Innovative Strategies for Agrochemical Safety Assessments: Use of Toxicokinetic Data for Arylex™ and Rinskor™

L. Murphy[2], S. Papineni[1], M. Aggarwal[1], M. J. Bartels[2] and R. Rasoulpour[1]. *[1]Dow AgroSciences, Indianapolis, IN and [2]The Dow Chemical Company, Midland, MI.*

Integrating toxicokinetics (TK) into toxicity studies, without use of additional animals, provides valuable data on metabolism, systemic exposure and dose response for observed toxicity. Guidance documents, such as the OECD GD 116, highlight the importance of TK for dose level selection. Increasingly, TK data are being used to provide insights on Mode of Action (MoA), study design, and *in vitro* to *in vivo* extrapolation for human health risk assessments. Described here are case studies, with the herbicides, Arylex™ (Halauxifen-methyl) and Rinskor™ (XDE-848 Benzyl Ester), highlighting the use of integrated TK approaches in novel testing strategies to explore a MoA, or implement kinetically-derived maximum dose (KMD) strategies, respectively. In the MoA case study, Halauxifen-methyl is rapidly hydrolyzed in rodent liver to a single primary metabolite, Halauxifen-acid, and induces rodent liver effects via nuclear receptor (NR) activation. Halauxifen-acid does not activate NR and *in vitro* assays in rat and human blood, liver S9, and gastric fluid evaluated species differences in hydrolysis rates. TK and hydrolysis data were incorporated into a PBPK model for rat and human systemic exposure to Halauxifen-methyl, and supported non-human relevance of the Halauxifen-methyl liver MoA. In the KMD case study, XDE-848 Benzyl Ester is hydrolyzed to a single primary metabolite, XDE-848 acid, which displays non-linear kinetics based on integrated TK from 28- to 90-day toxicity studies. Interestingly, these studies indicated no toxicity up to the limit dose (1000 mg/kg/day) and the TK data provided strong justification for use of a KMD approach on the OECD 453 and 416 studies in the rat, and 1-year chronic toxicity study in the dog. A high dose of 300 mg/kg/day was chosen in contrast to traditional toxicity testing paradigms using a maximum tolerated dose approach where the limit dose is chosen as the high dose in the absence of toxicity. Taken together, integration of TK into guideline toxicity studies can increase understanding of the intrinsic properties of a molecule to develop human health risk assessments that utilize the best available science.

2459 Inhalation Health Effects Testing of Isobutanol Gasoline Blend: A Promising New Biofuel

D. Burnett[1], J. Baustian[2], G. Hoffman[4], R. Parker[4] and J. O'Callaghan[3]. [1]*BP, Global Product Stewardship, Naperville, IL;* [2]*Butamax Advanced Biofuels, Wilmington, DE;* [3]*CDC-NIOSH, Morgantown, WV and* [4]*Envigo, East Millstone, NJ.*

Isobutanol is a high-value molecule offering several advantages over existing transportation biofuels. Isobutanol blended with gasoline at 16% is currently under development as a new automotive biofuel in the US. To evaluate the potential health effects from exposure to isobutanol gasoline blend when dispensed at the pump, a battery of GLP whole-body inhalation studies using Sprague-Dawley rats was conducted in accordance with US EPA fuel testing requirements, described in Section 211(b) of the Clean Air Act and its Alternative Tier 2 Rule. Testing included the following guideline studies: 90-day subchronic toxicity with neurotoxicity, neuropathology, glial fibrillary acidic protein (GFAP) measurements across 8 brain regions, and 4-weeks recovery; 28-day immunotoxicity; 28-day genotoxicity; one-generation reproductive toxicity; and embryo-fetal developmental toxicity. To approximate the hydrocarbon composition of fugitive emissions to which humans are potentially exposed during refuelling, light-end hydrocarbon emissions were generated from the whole fuel heated to 130°F and condensed to form a vapor condensate (VC) test substance. Isobutanol levels in the VC were 2–3%. In all studies rats were exposed to re-vaporized test substance at target concentrations of 0 (air control); 2,000; 10,000; and 20,000 mg/m3, the latter being 50% of the lower explosive limit of the VC. Exposure frequency and duration were tailored to study design. The only treatment-related finding in the 90-day study was light hydrocarbon nephropathy in male rats, a known effect specific to male rats and not relevant to humans. No treatment-related findings were seen in neuropathology, GFAP, neurotoxicity, immunotoxicity, genotoxicity, or developmental toxicity. The no-observed-adverse-effect level (NOAEL) for these endpoints was 20,000 mg/m3. In the reproductive study transient changes in body weight gain and food consumption were seen in high-dose paternal and maternal animals. These effects occurred at hydrocarbon exposure levels several orders of magnitude above conditions typically experienced at dispensing pumps during refueling (<1 mg/m3, 5 min). Based on these results, inhalation exposure to light-end hydrocarbons emitted from 16% isobutanol gasoline during refuelling is unlikely to present a unique health hazard to humans.

2460 A New E-Cigarette Aerosol pH Technique with Improved Toxicological Relevance

J. H. Lauterbach and S. J. Lauterbach. *Lauterbach & Associates, LLC, Macon, GA.*

In the past, the determination of pH-values of mainstream cigarette smoke was controversial both in terms of analytical methodology and interpretation of the results in terms of nicotine addiction and other toxicological effects. The controversy has continued with the introduction of e-cigarettes (e-cigs) and related devices and the e-liquids (e-liqs) used with them, but there is one main difference. The aerosol generated by e-cigs does not contain carbon dioxide and other acids generated from combustion of the tobacco. Since there are no products of combustion, e-liq pH should equal e-cig aerosol pH. This is not the case. E-liquids contain little or no water, and must be diluted with water to achieve the dilute aqueous solution needed for accurate pH measurements and possible use of the Henderson-Hasselbalch equation. However, dilution of mentholated and highly flavored e-liquids results in cloudy mixtures. Attempts to measure the pH on undiluted e-liq is technically incorrect as e-liquids pick up water from ambient drawn in by users puffing on the devices, sometimes increasing water concentration as high as 10%, and there may be precursors of volatile acids in e-liquids that are activated by the e-cig heater assembly. Thus, pH determinations should be conducted on the undiluted aerosol emitted by the e-cigs. Health Canada Test Method T-113 has been used for e-cig aerosols, but T-113 specifies a modified pH electrode and sampling chamber that is atypical of the human mouth. These disadvantages can be overcome by using nonstandard pH electrode is a glassmouth such that the active portion of the electrode is in the smoke stream. One example system is based around a Hanna Instruments HI-99171N Leather and Paper pH Meter and probe. Without an aerosol stream, resting pH ≈7, with the aerosol (4-sec 55-mL puff every 30 sec) from popular brand 1, pH ≈7.2 and from popular brand 2 (known to contain organic acid), pH ≈6.8; nicotine at 50 mg/mL in PG, pH ≈7.6. These values are lower than obtained with other techniques and indicate that likelihood of adverse health effects from high pH may have been overestimated. Placement of water or synthetic saliva in bottom of glassmouth permits a second check on the pH values and change in water and/or saliva pH during and after vaping.

2461 Microbiological Prevention in the Concentration of Bacterial Pathogens on the Athletic Equipment in the Heal Complex at Paine College

M. J. Stephens, J. R. Johnson and G. J. Swenson. *Department of Mathematics, Sciences and Technology, Paine College, Augusta, GA.* Sponsor: T. Burns.

Background: *Staphylococcus aureus* is a gram positive bacterium and a commonly acquired hospital pathogen. *S. aureus* is normally found in moist, warm areas of the human body such as armpits, crotches, and the upper respiratory tract. Delayed detection of *Staphylococcus aureus* can lead to a more severe infection known as Methicillin resistant *Staphylococcus aureus* (MRSA). MRSA was originally found in hospitals or health care facilities. However, MRSA has now branched to the community, known as Community Associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA). MRSA is a difficult infection to treat because of its resistance to antibiotics of the penicillin family. If this infection is not treated, it can become systemic and lead to organ failure. Recent reports show that *Staphylococcus aureus* was present on the frequently used athletic equipment utilized by students and athletes on campus. Methods: Research was conducted in the HEAL Complex to monitor changes in *S. aureus* concentrations in the athletic weight room after a MRSA bundle (informative presentation and signage postings throughout weight room) was presented to the athletic department in an effort to raise awareness about risk of infection. Over seven sample periods, athletic equipment was swabbed using sterile cotton swabs. After being re-suspended in 10% sterile saline, they were subcultured in triplicates for each equipment piece. Samples were grown on Mannitol Salt Agar and incubated for 3 days at 37ºC. Colonies were counted. Results: Some athletic equipment such as the treadmill, incline, bench, and crunch saw a sharp decrease in *S. aureus* concentrations over the sample period. However, the biceps equipment had an overall increase. While not a strong linear relationship (R2=0.01), a downward trend in *S. aureus* concentrations was detected following the MRSA bundle presentation. Conclusion: The results of this project suggest that the introduction of a MRSA bundle was successful in decrease the concentrations of *Staphylococcus aureus* athletic equipment.

2462 Identification of Compounds That Modulate Retinol Signaling Using a Cell-Based qHTS Assay

Y. Chen[1], S. Sakamuru[2], R. Huang[2], D. H. Reese[1] and M. Xia[2]. [1]*Center for Food Safety and Applied Nutrition, US FDA, Laurel, MD and* [2]*National Center for Advancing Translational Sciences, NIH, Bethesda, MD.*

all-trans Retinoic acid (atRA) is the most abundant natural metabolite of retinol (vitamin A). Physiological levels of atRA are maintained by the retinol signaling pathway (RSP), which controls the biosynthesis and catabolism of atRA. atRA regulates transcription of genes that are essential for embryonic development. Chemicals that interfere with the RSP can cause abnormal intracellular levels of atRA and therefore are potential developmental toxicants. To assess chemicals for the potential to adversely affect embryonic development by interfering with retinol signaling, we have developed a cell-based RARE (Retinoic Acid Response Element) reporter gene assay that can be used to identify disruptors of the RSP. To validate the RARE assay in a quantitative high-throughput screening (qHTS) platform, we screened the Library of Pharmacologically Active Compounds (LOPAC) in both agonist and antagonist modes. The screens detected known RSP agonists, demonstrating assay reliability. In addition, the screens identified novel RSP agonists including kenpaullone, niclosamide, PD98059 and SU4312, and RSP antagonists including Bay 11-7085, LY294002, 3,4-Methylenedioxy-β-nitrostyrene (MNS), and a set of topoisomerase inhibitors (camptothecin, topotecan, amsacrine hydrochloride, and idarubicin). These compounds were further evaluated in the P19 pluripotent cell and were found to affect the expression of the Hoxa1 gene that is essential for body patterning during development. These results show that the RARE assay is an effective qHTS approach for screening large compound libraries to identify chemicals that have the potential to adversely affect embryonic development through interference with retinol signaling.

2463 A Biology Based Approach for Chemical Grouping Using Metabolomics

H. G. Kamp[3], B. van Ravenzwaay[3], E. Fabian[3], E. Peters[1], G. Krennrich[3], W. Mellert[3], V. Strauss[3] and T. Walk[1]. *[1]metanomics GmbH, Berlin, Germany; [2]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany and [3]Quantum Chemistry, BASF SE, Ludwigshafen am Rhein, Germany.*

BASF has developed a very large metabolomics data base (MetaMap®Tox) containing >500 data rich chemicals, agrochemicals and drugs based on 28 day studies in rats with blood metabolic profiling at day 7, 14 and 28 of treatment. More than 100 specific metabolome patterns have been established which are associated with different toxicological modes of action. In addition whole metabolome comparison of compounds with all compounds in the data base provides a ranking list of overall bets matches. Here we present three examples using metabolomics to demonstrate how the quality of chemical grouping for read across purposes, an important procedure to reduce animal testing under the REACH legislation, can be improved: 1. Mesotrione and Topramezone are herbicidal active ingredients, inhibiting the HPPD enzyme. NTBC is a drug with the same mode of action. Their common metabolome profile consist of 11 regulated metabolites. This profile allowed for the identification of another HPPD active compound. Moreover, the 4th compound ranked excellently with other HPPD inhibitors. 2. Fibrates are drugs used for LDL-cholesterol lowering effect. The metabolome of three fibrates (Beza-, Clo-, Fenofibrate) was evaluated (liver as target organ). The metabolome of a fourth fibrate (gemfibrozil) revealed an additional toxic effect on kidneys and a different cholesterol lowering effect compared to the above mentioned fibrates. 3. Finally, we could also demonstrate that structurally similar compounds (2-AAF and 4-AAF), which have toxicologically very different profiles (2-AAF as liver carcinogen, but not 4-AAF), also are different from a metabolome perspective. In the compound comparison procedure 4-AAF was only found on rank 1416 (out of 1907) when compared to 2-AAF. 2-AAF was identified as toxic to the liver but not 4-AAF. The combined evaluation of specific mode of action patterns with chemical ranking based on the entire finger print is a powerful tool for biology based grouping of chemicals. Going from QSAR to QBAR (quantitative biological activity relationship).

2464 The Evaluation of the Safety of Self-Assembling Peptide Gel After the Intracranial Administration to Rats by Using an Open Field Test

M. Tsunoda[2], C. Sugaya[2], Y. Sugiura[2], Y. Nagai[1] and K. Sakanishi[1]. *[1]Menicon, Co., Kasugai, Aichi, Japan and [2]Hygiene, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan.*

Self-assembling peptides have been employed in various tissue engineering studies. Regarding the clinical application of self-assembling peptides, it is expected that they will be used for scaffolds for implanted cells and hemostatic materials. To our knowledge, there are no studies on the effects of self-assembling peptides on the central nervous system. If the safety of self-assembling peptides for the clinical local application for the brain is confirmed, they will be used as scaffolds to regenerate nerves and hemostatic materials in the brain. In this study, the safety of self-assembling peptides for the application on the brain was examined by the open field test of rats that had the self-assembling peptides applied to the cerebrum. A circular disk of bone 5 mm in diameter was cut from the cranium of each anesthetized rat using a system composed of a drill and micromotor with a 5 mm in diameter bone trephine bar. For the peptide gel group, 20 µl of 1.5% of the self-assembling peptide gel was administered to the surface of the brain. The control group underwent a sham surgical operation. As the positive controls, 20 µl of TBT chloride was administered on the brain at the concentration of 10 µg/ml. After 2 months, the open field test was carried out for each rat. The mean value of locomotor distance from 20 to 25 minutes in the TBT-administered group in the open field test was significantly lower than that in the control. There were no significant differences between the peptide gel group and the control group in the locomotor distances. However, the mean value of the percentage of time the rats stayed in the central area of the open field in the peptide gel group was significantly higher than that in the control. These increases in time the rats stayed in the central area and the instance of face washing in the open field were not considered to be toxic effects. Under this protocol, the application of self-assembly peptides on the cerebrum did not induce neurotoxic effects. The clnical application of self-assembly peptides on the brain is possible. As the positive control for intracranial administration of chemical substances, TBT at the dose used in this study is adequate.

2465 Application of Dermal Absorption Values to Refine Margin of Safety Calculations in EU Cosmetics Safety Assessments

M. Boylan, J. Fleischer and M. Whittaker. *ToxServices LLC, Washington, DC.*

As required by EC Regulation No. 1223/2009, cosmetics in the European Union must be safe for their intended use. This safety is established in part through a Margin of Safety (MOS) calculation, which is obtained by dividing the most relevant toxicity factor by the systemic exposure dosage (SED). MOS values of 100 are considered a sufficient margin of safety for most cosmetic ingredients. MOS calculations assume complete (100%) dermal absorption of an ingredient as a default in cosmetic safety. However, this assumption often results in unacceptable MOS values for ingredients with a long history of safe use in cosmetics. The following data sources can be used to refine dermal absorption values to more accurately estimate absorbed dermal doses: *in vitro* dermal absorption studies, measurement of volatilization of chemicals from skin, analysis of monomer absorption, and the measurement of lipophilicity. For example, a calculated MOS value for aminomethyl propanol was 0.82, which does not demonstrate safety of this ingredient at its proposed use level in a cosmetic. An *in vitro* dermal absorption study resulted in a DAF of 6.6% when a lotion containing 4% aminomethyl propanol was applied to excised human skin for 24 hours. Application of this DAF to the SED resulted in a MOS of 123, which substantiated the ingredient's safe use in a cosmetic. In a second case, an MOS value of 57 was derived for sodium acrylates copolymer based on an oral NOAEL of 83 mg/kg/day. As sodium acrylates copolymer is assumed to have limited dermal absorption potential due to its large molecular weight, no dermal absorption studies have been conducted. This necessitates an alternate, yet still conservative, approach to refine the SED. The polymer's acrylic acid monomer can be absorbed dermally; however, most volatilizes from the skin. In a dermal absorption study in rats, 73% volatilized and 24% remained in exhaled air, at the dosing site, and in tissues. Therefore, the SED was adjusted using a DAF of 24%, resulting in an acceptable MOS of 239. This demonstrates the utility of dosimetric adjustment factors as part of EU cosmetic safety assessments.

2466 Successful Application of Skin Sensitization Quantitative Risk Assessment for Hair Dyes Using Skin Penetration Measurements

H. Rothe[2], G. F. Gerberick[1] and C. Goebel[2]. *[1]Central Product Safety, The Procter & Gamble Company, Mason Business Center, OH and [2]Central Product Safety, The Procter & Gamble Company, Schwalbach am Taunus, Germany.*

The *in vitro* skin penetration assay was developed primarily as a tool to quantify the amount of an ingredient penetrating through the skin after dermal application resulting in a systemic exposure. However, the assay provides a good platform to quantify not only what went through the skin and was systemically absorbed, but also what stays on and in the skin. As the *in vitro* skin penetration assay allows application of product under in use exposure conditions like correct product formulation or number of product uses as well as rinsing patterns, one can use this assay to measure the actual consumer exposure level (CEL), rather than estimating exposure based on conservative assumptions. This measured CEL (MEL) can be used directly as input in a Quantitative Risk Assessment (QRA) to be compared with the threshold level for the induction of skin sensitization derived from historical animal and/or human data, i.e., the non expected sensitization induction level (NESIL). For p-phenylenediamine (PPD), a key molecule for hair dye products with strong skin sensitizing properties, the NESIL and the MEL are in the same order of magnitude with the NESIL only slightly above the MEL. This relation indicates that the majority of consumers do not acquire skin sensitization to PPD through hair coloring, while inter-individual variability is not sufficiently covered to exclude skin sensitization induction at maximal use concentrations. This reflects the current market observation and is supported by a recent European multi-center epidemiology study (Diepgen et al., 2015), demonstrating no relevant difference for PPD allergy between users and non-users of hair dyes in the general population. Reference: TL Diepgen, L Naldi, M Bruze, S Cazzaniga, ML Schuttelaar, P Elsner, M Goncalo, R Ofenloch, A Svensson. Prevalence of contact allergy to p-phenylenediamine (PPD) in the European general population. J. Invest. Dermatol., accepted for publication, August 2015

2467 Evaluation of the Chemical Inventories in the US FDA's Office of Food Additive Safety for Human Health Endpoints Using a Toxicity Prediction System

K. Arvidson[5], P. Volarath[5], A. Mostrag[1], B. Bienfait[2], A. Tarkhov[2], V. Vitcheva[1], J. Rathman[1,3] and C. Yang[1,2]. *[1]Altamira LLC, Columbus, OH; [2]Molecular Networks GmbH, Erlangen, Germany; [3]Ohio State University, Columbus, OH; [4]US FDA, College Park, MD and [5]Office of Food Additive Safety, US FDA, College Park, MD.*

The Chemical Evaluation and Risk Estimation System (CERES) at the US FDA's Office of Food Additive Safety (OFAS) implemented the ChemTunes prediction system within the workflows for pre-market reviews and post-market monitoring of food ingredients and packaging materials. A set of 2474 structures was compiled from diverse data sources at OFAS, including EAFUS, FCS, FEMA, PAFA, and SCOGS lists. The chemical space of the resulting inventory was profiled using ToxPrint, physicochemical properties, and toxicity potentials. The human health related endpoints included the full genetic toxicity battery, rodent tumorigenicity, target organ and repro/developmental toxicity, and skin sensitization. Predictions were based on both chemotype alerts and mode-of-action informed QSAR models by applying a quantitative weight of evidence (WOE) method. Prediction accuracies were validated by experimental data. The domain of applicability of models and chemotype alerts were systematically addressed. Based on the WOE predictions, approximately 4-5 % of the structures in the combined inventory are considered positive in at least one mutagenicity or one clastogenicity endpoint. A total of 179 chemicals were predicted to be tumorigens; about half were predicted positive in both Ames and clastogenicity assays (*in vivo* or *in vitro*), the other half being non-genotoxic tumorigens. Profiling the structures using chemotype alerts gives 3% hits for reproductive/developmental alerts, 5% for mitochondrial toxicity alerts, and 12% for liver steatosis alerts. This work demonstrates how post-marketing evaluation based on a profiling analysis of the historical inventories provides opportunities to enhance the current workflow by applying the advanced methods in the ChemTunes knowledgebase within CERES. Reference to ChemTunes does not constitute an endorsement by US FDA.

2468 Evaluating the Ability of High-Throughput Screening (HTS) Assays to Capture the Biological Activity of Industrial Chemicals

K. O. Goyak[2], R. Alyea[2], R. A. Becker[1], L. M. Plunkett[3] and J. B. Plunkett[3]. *[1]American Chemistry Council, Washington, DC; [2]ExxonMobil Biomedical Sciences, Inc., Annandale, NJ and [3]Integrative Biostrategies LLC, Houston, TX.*

In 2013, Thomas et al. (Toxicol Sci 136:4-18) proposed a data-driven framework to encourage the use of non-traditional data in safety assessments. The first step of this data-driven framework is analysis via a broad set of HTS assays to separate chemicals into 2 categories: those acting nonselectively (i.e., nonspecific biological response pathways (BRPs)) & those eliciting selective BRPs (e.g., receptor-mediated effects); activation of >20 *in vitro* gene targets (of 322 available unique gene targets) would indicate a nonspecific BRP and <20 a selective BRP. To better understand coverage of HTS assays relevant to toxicity of industrial chemicals, we compared *in vivo* effects & *in vitro* "actives" for chemicals that have been evaluated in both IRIS & ToxCast (n=214). Using the effect underlying the oral reference dose (RfD), 36 effect categories were assigned. For the 214 IRIS chemicals, the most often assigned effect categories were liver (63), body weight changes (44), & kidney (34); 11 categories contained 10 or more chemicals. We hypothesized that chemicals assigned a specific target organ designation were acting via a selective BRP and chemicals assigned a more general effect (e.g., body weight change) would act via a nonspecific BRP. The majority of chemicals in 5 of 11 effect categories were correctly predicted as selective or nonspecific based on the *in vitro* gene targets (selective: kidney, developmental, male reproductive; nonspecific: clinical chemistry changes, clinical observations). The remaining 6 categories were not correctly predicted (e.g., body weight changes were considered a nonspecific response yet 24/44 chemicals activated <20 gene targets). Analysis of actives within the correctly-predicted categories might provide additional biological context for the associated assays & increase confidence for future use. Specific outcomes (e.g., peripheral neuropathy) that were not identified as selective in this analysis could be targeted for mechanistic assay development.

2469 Sex Specific Differences in Repeat-Dose Toxicity in Rats After Treatment with n-Butyronitrile

S. M. Green. *Eastman Chemical Company, Kingsport, TN.* Sponsor: S. Green.

n-Butyronitrile (NBN) is used primarily as a process solvent and a chemical intermediate. In the past, NBN has not undergone repeat-dose testing due to its high level of acute toxicity, which mandates significant personal protective equipment and engineering controls and normally preclude repeat exposure. Under the REACh chemical registration program in Europe, such repeat dose testing is mandatory for non-intermediates; thus, due to its use as a solvent, NBN is under test orders for 90-day oral repeat dose and pre-natal developmental toxicity studies in rats. Range-finding studies were initially performed as the cumulative effects of NBN are unknown. Repeat dose toxicity was evaluated by a 14-day range-finder in both male and female rats that was extended to 75 days due to high levels of toxicity. Doses of 25, 50, 100 and 250 mg/kg/day were used. Males exhibited prominent toxicity as measured by mortality, decreased body weight gain and outright weight loss. The 250 mg/kg dose group was lost by day 7, and the 100 mg/kg group by day 49. For the remaining dose groups, the percent difference in body weights versus control increased over the dosing period, although the males were gaining weight over the dosing period. By the end of dosing on day 75, the 50 mg/kg/day male body weight was 15.6% lower than the controls, while the 25 mg/kg/day group was 8.3% lower. In females, the 250 mg/kg dose group was lost by day 14, but all other dose groups maintained a normal body weight, and at almost every day measured, their body weights exceeded the control. Necropsy evaluations were limited due to the study design, but findings include anemia, increased reticulocytes, increased liver enzymes (ALT, ALP) and organ weight changes in the liver, kidney, heart, spleen and adrenal glands. In a range-finding developmental study, similar doses produced a primary difference in the 100 mg/kg/day group where the test article was not tolerated well, and body weight gains were not maintained. The primary developmental effect was litter resorption in 6/8 rats at the high dose of 100 mg/kg/day.

2470 Re-Shaping Acute Toxicity Testing of Agrochemical Formulations by Combining the GHS ATE Formula and *In Vitro* Approaches

M. Corvaro[1], S. Gehen[2], K. Andrew[1], C. Arasti[1], R. Chatfield[1], F. MacLeod[1], H. Mikolajczak[1], J. Moore[1] and J. Mehta[1]. *[1]Dow Agrosciences, Abingdon, United Kingdom and [2]Dow Agrosciences, Indianapolis, IN.* Sponsor: D. Juberg.

An acute toxicology six-pack of studies is currently required for the registration and Classification and Labelling (C&L) of agrochemical formulations. The UN GHS (Globally Harmonised System for C&L) provides the opportunity to calculate the classification category based on the Acute Toxicity Estimate (ATE) of each individual component within the mixture, without performing additional *in vivo* studies. A retrospective analysis was performed for more than 200 agrochemical formulations in order to A) identify main classification drivers and prioritise research on methods alternative to *in vivo* testing; B) evaluate accuracy of the GHS ATE approach, comparing to data from existing *in vivo* toxicity studies. For the acute systemic toxicity end-points (oral, dermal and inhalation), the main hazard concern was acute oral toxicity (despite being less relevant from an exposure perspective). A classification threshold of 2000 instead of 5000 mg/kg bw should be used for agrochemical formulations. The GHS ATE formulae showed high accuracy and specificity (both > 85% and up to 99.5%) for prediction of the estimated LD50 compared to 4 classification systems GHS, CLP, EPA and ANVISA. Using the GHS ATE formula together with physico-chemical characteristics related to exposure as a criterion for triggering the systemic toxicity study, would lead to a potential animal use reduction of up to ~48% (currently implemented in limited geographies). For the topical effects (skin/eye irritation; skin sensitisation), variability of the reference *in vivo* dataset was highlighted also for agrochemical formulations (see Adriaens et al., 2014). Predictions using the GHS ATE formulae resulted in an overall accuracy of ~70%. However, combination of GHS ATE with results from accepted *in vitro* methods and the use of Adverse Outcome Pathways (AOP) provides the opportunity to move beyond animal testing. Taken together, the combination of ATE, AOP and *in vitro* tools provides a reliable, animal-free and alternative to traditional acute toxicity testing.

2471 Development of an *In Vitro* Test for Allergenic Potency of Proteins

H. Tsukumo[3], N. Matsunari[3], M. Sugiyama[2], A. Toyoda[2,3], H. Kojima[1] and H. Itagaki[3]. [1]*National Institute of Health Sciences, Setagaya, Japan;* [2]*Pola Chemical Industries, INC., Yokohama, Japan and* [3]*Yokohama National University, Yokohama, Japan.*

Introduction: Proteins are common ingredients of both foods and non-foods, including cosmetics and toiletries. Proteins have a high molecular weight, due to which they have not conventionally been considered readily absorbable through the skin. There have, however, been recent cases reported in Japan of patients who had initially been sensitized to hydrolyzed wheat protein from facial soap and later experienced wheat-dependent anaphylaxis after ingesting normal wheat products. It is, therefore, considered very important that the skin-sensitizing potency and allergenic potency of proteins be assessed. Moreover, due to considerations related both to animal welfare and differences between humans and animal species, the development of an *in vitro* test is desirable. Given this background, we have developed a test for protein-induced skin sensitization based on the human Cell Line Activation Test (h-CLAT). Materials and Methods: We modified the h-CLAT method and adopted the same criteria. THP-1 cells (1×106 cells/mL) were treated with 0.1, 1.0, and 10.0 mg/mL of each protein and cultured for 24 hours. The cells were stained with antibodies, and the fluorescent intensity of the cell surface markers were analyzed by flow cytometry. We used seven proteins in total, including ovalbumin (OVA). Results and Discussion: Both CD86 and CD54 expression of cells treated with OVA exhibited augmentation that exceeded the criteria. Six of the seven proteins were judged to be positive and one to be negative. In summary, our findings show that THP-1 responds not only to chemicals but also to proteins, and we feel that this test could be useful as an *in vitro* test for assessing the skin-sensitizing potency of proteins. Now, we investigate the mechanism of interaction to proteins. In the future, we plan to test more proteins and optimize the test parameters as well as to develop an *in vitro* test for assessing the allergenic potency of proteins.

2472 A Generic PBPK Model for Assessing *In Utero* Toxicokinetics: Application to Bisphenol A

D. Sarigiannis[1,2,3], S. Karakitsios[1,2], A. Gotti[1,3] and E. Handakas[1]. [1]*Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*Chemical Process and Energy Resources Institute, Centre for Research and Technology Hellas, Thessaloniki, Greece and* [3]*Environmental Health Engineering, Institute for Advanced Study, Pavia, Italy.* Sponsor: G. Miller.

The lifetime generic PBTK model presented herein is designed to describe mother fetus interactions by modelling the intra-placental properties that govern the transfer of xenobiotics and their metabolites from the mother to the fetus as the latter grows *in utero*. The anthropometric parameters of the models are time dependent, so as to provide a lifetime internal dose assessment, as well as to describe the continuously changing physiology of the mother and the developing fetus. For assessing fetal exposure to bisphenol A (BPA), a maternal overall BPA daily intake equal to 0.5 μg/kg_bw/d was assumed. In this case, free plasma BPA in maternal blood is almost 0.002 μg/L, i.e. slightly higher than the one expected for a non-pregnant woman (0.0016 μg/L). Placental concentration is 0.004 μg/L and the corresponding fetal free plasma BPA concentration is 0.0015 μg/L. Although placenta has almost twice the concentration of free BPA compared to the other maternal tissue compartments, fetal concentration is lower because the whole fetus acts as a compartment with limited metabolic capacity, due to the presence of active enzymes able to sulfate BPA. Had sulfation been negligible, the fetus would have be an additional non-metabolizing compartment to the overall fetus - placental system, and the steady state concentration would be slightly higher (around 0.0025 μg/L) than the maternal one, yet lower than the placental concentration. Thus, free plasma BPA in the fetus is sensitive to the extent to which sulfation develops, as well as to the effect of β-glucuronidase in the placenta that results in BPA-Glu deconjugation. If the effect of β-glucuronidase was negligible, then the maternal concentration should be 0.0015 μg/L, which is slightly lower than the one of a non-pregnant woman. Placental concentration would be even lower (0.0013 μg/L) and the corresponding fetal exposure would be 0.0005 μg/L, a value that is not consistent with experimental data.

2473 Evaluation of an Aqueous Extract of *Terminalia chebula* for Anti-Arthritic Efficacy and Safety in Osteoarthritic Dogs: Radiographic Evidence

N. Murdock[1], R. C. Gupta[1], N. Vega[1], K. Kotora[1], J. F. Miller[1], J. T. Goad[1], M. A. Lasher[1], T. D. Canerdy[1] and S. R. Kalidindi[2]. [1]*Murray State University, Murray/Hopkinsville, KY and* [2]*Natreon Inc., New Brunswick, NJ.*

Currently, ~ 20% of the adult and 80% of geriatric dog population in the US (>80 million) suffer from osteoarthritis (OA). The present investigation was carried out to assess anti-inflammatory and anti-arthritic properties and safety of a standardized aqueous extract of *T. chebula* (TCE) in moderately OA dogs. Dogs with OA received either 500 mg placebo or 500 mg TCE b.i.d. for 150 days. Each month, dogs were given a full physical exam and were evaluated for arthritic pain using different criteria, joint flexibility using goniometer, CBC and erythrocyte sedimentation rate (ESR), and serum biomarkers of liver (bilirubin, ALT, and AST), kidney (BUN and creatinine), and heart and skeletal muscle (CK) functions. Elbow and stifle joints were radiographed on day 0 and day 150 for evaluation of osteophyte formation and cartilage damage. Dogs given TCE showed significant ($P<0.01$) reductions in overall pain, pain upon limb manipulation, and pain after physical exertion by 60–90 days, with maximum effect on day 150. A marked reduction in ESR coincided with pain reduction in treated dogs, which was indicative of anti-inflammatory effect of TCE. Radiographic evidence also indicated a marked decrease in osteophyte formation and cartilage damage. No significant change occurred in physical parameters, serum biomarkers or CBC parameters in dogs on placebo or treatment, which suggested that TCE was well tolerated. It can be concluded that T. chebula extract, by having many active principles, such as chebulagic acid, chebulinic acid and low molecular weight hydrolysable tannoids, might have provided anti-inflammatory and anti-arthritic effects in dogs without causing any side effects.

2474 Integrated Testing Strategies (ITS) for Choosing Surfactants and Preservatives Applied to Children's Cosmetic Products

A. D. Canavez[1], G. A. Cararo[1], B. B. Swinka[1], A. C. Weihermann[1], C. R. Neumann[1], C. Sasson[1], O. Kruger[1], C. Brohem[1], R. Wielecosseles[1], H. Maibach[2] and M. Lorencini[1]. [1]*Boticario Group, São José dos Pinhais, PR., Brazil and* [2]*Department of Dermatology, University of California San Francisco, California, USA, Brazil.* Sponsor: H. Maibach.

Safety is one of the most important factors in the buying decision of children's cosmetic products. In the specific case of shampoos, the analysis of ocular irritation potential and the correct choice of ingredients are essential to ensure consumer safety. The aim of this study was to integrate *in vitro* and clinical tests to support the choice of safe ingredients for the formulation of children's shampoos, minimizing possible eye irritations. Following the three R's principle, the *in vitro* methodology of agar diffusion with SIRC cells was developed and the EpiOcular technique was conducted to ensure volunteers' safety before clinical exposure. Additionally, an *in vivo* test was performed to evaluate eye irritation, burning and/or lacrimation after product instillation and under ophthalmological supervision. A basic formulation was chosen and three different preservative systems were tested separately (each one at an effective antimicrobial concentration): 1) Benzyl Alcohol and Caprylyl Glycol; 2) Sodium Benzoate; and 3) Caprylyl Glycol. The lowest irritant potential was observed for the mixture of Benzyl Alcohol and Caprylyl Glycol, which was applied to three new formulations with different surfactant compositions and resubmitted to the same *in vivo* tests. The best results were observed for formulations enriched with Glucoside or Cocoyl Isethionate surfactants. The combination of different methodologies showed to be efficient for the best choice of preservatives and surfactants in the development of safe children's cosmetic products.

2475 Final Report on the Development of Non-Cancer Threshold of Toxicological Concern (TTC) Database to Support Alternative Assessment Methods for Cosmetics-Related Chemicals

H. M. Hollnagel[3], K. Arvidson[9], S. Barlow[2], A. Boobis[6], M. T. Cronin[7], S. P. Felter[8], D. Keller[5], K. L. Muldoon Jacobs[9], V. Vitcheva[1], A. P. Worth[4] and C. Yang[1]. *[1]Altamira LLC, Columbus, OH; [2]Consultant, Brighton, United Kingdom; [3]Dow Europe GmbH, Horgen, Switzerland; [4]EC Joint Research Center, Ispra, Italy; [5]Henkel, Düsseldorf, Germany; [6]Imperial College, London, United Kingdom; [7]Liverpool John Moores University, Liverpool, United Kingdom; [8]P&G, Cincinnati, OH and [9]US FDA CFSAN OFAS, College Park, MD.*

Regulatory drivers are requiring that alternative methods to animal testing be used in the assessment of cosmetic ingredients and formulations. The threshold of toxicological concern (TTC) is one useful approach to estimate potential systemic toxicity of compounds with limited toxicology data. The COSMOS consortium within SEURAT-1 has developed a new non-cancer TTC database enriched with cosmetic-related chemicals. The sources of the toxicity data for the TTC database have been primarily of regulatory origin, including EU SCCS, EFSA, US FDA, US EPA along with the technical reports from US National Toxicology Program. This has been augmented with chemicals from the Munro dataset found in Cosmetics Inventories. Studies were selected according to a strict set of inclusion criteria. The resulting TTC database contains information on over 556 chemicals, including NOAELs and critical effects at the LOAELs. Over 25% of the toxicity data were evaluated in four study review sessions by an ILSI Europe Expert Group, including studies for the lowest 10th percentile of NOAELs from each Cramer Class, and studies showing large discrepancies across the data sources. The chemical space of the new TTC database has been compared with that of other existing TTC databases, demonstrating its suitability of coverage for the assessment of cosmetic-related chemicals. Comparing the 5th percentile values of the distributions of NOAELs in the COSMOS TTC database with those from the Munro database showed that existing TTCs values are adequately protective. This abstract does not reflect the policy of US FDA, JRC, or EC.

2476 COSMOS DB as an International Share Point for Exchanging Safety Evaluation and Toxicity Data, and Expanding the Known Chemical Space

M. Cronin[5], C. Yang[1,6], A. Bassan[10], K. Arvidson[8], I. Boyer[2], E. Fioravanzo[10], B. Heldreth[2], J. Kim[4], J. Madden[5], J. Rathman[1,9], C. Schwab[6], T. Yamada[7] and A. Worth[3]. *[1]Altamira LLC, Columbus, OH; [2]Cosmetic Ingredient Review, Washington, DC; [3]EC Joint Research Centre, Ispra, Italy; [4]Foundation of Korea Cosmetic Industry Institute, Osan, Korea, Republic of; [5]Liverpool John Moores University, Liverpool, United Kingdom; [6]Molecular Networks GmbH, Erlangen, Germany; [7]National Institute of Technology and Evaluation, Tokyo, Japan; [8]Office of Food Safety, US FDA CFSAN, College Park, MD; [9]Ohio State University, Columbus, OH and [10]S-IN, Vicenza, Italy.*

The COSMOS database (DB) was established in 2013 as a publicly available cosmetics inventory and source of repeated dose toxicity data for cosmetics-related chemicals. Version 2.0 now includes a safety evaluation section to incorporate various Threshold of Toxicological Concern (TTC) datasets. The ultimate goal of the consortium is to enable method development for the eventual replacement of animal-based repeated-dose toxicity and biokinetics testing of cosmetic ingredients and consumer products. To this end, the COSMOS DB captures data from the Scientific Committee of Consumer Safety and European Food Safety Authority and has also catalyzed the data exchange among numerous international institutions, including the US FDA CFSAN, the Hazard Evaluation Support System (HESS) through National Institute of Technology and Evaluation of Japan, the Cosmetic Ingredient Review (CIR), and the Korea Cosmetic Industry Institute (KCII). The database also houses the critical point of departure data identified by the COSMOS TTC project in collaboration with the ILSI Europe, as well as other TTC datasets such as those established by Munro et al. (non-cancer) and CPDB (cancer). The COSMOS DB thus functions as the de facto clearing house for TTC datasets, which can be exported from the database. This presentation launches the next generation COSMOS beyond the SEURAT-1 initiative and demonstrates the power of COSMOS DB as an international share point in a variety of safety assessment cases. This abstract does not reflect the policy of US FDA, CIR, JRC, EC, NITE, or KCII.

2477 A Four-organ-on-a-chip Microfluidic System: Towards a Tool for Long-term Systemic Toxicity Assessment

A. Lavado[4], C. Oleaga[4], S. Rothemund[4], Y. Cai[4], L. Kumanchik[4], L. R. Bridges[4], C. Martin[4], M. Jackson[4], C. W. McAleer[4], C. J. Long[4], J. Langer[2], A. Riu[2], R. Note[2], S. Teissier[1], J. Cotovio[1] , L. Breton[1], M. L. Shuler[3] and J. J. Hickman[4]. *[1]L'Oreal Research and Innovation division, Aulnay-sous-Bois, France; [2]L'Oreal Research and Innovation division, Clark, NJ; [3]Biomedical Engineering, Cornell University, Ithaca, NY and [4]NanoScience Technology Center, University of Central Florida, Orlando, FL.* Sponsor: E. DuFour.

Concerns about long-term repeat-dose toxicity assessment have been raised since the ban on animal testing for the cosmetic industry. Hence, the generation and characterization of alternatives *in vitro* systems capable of reproducing the functionality of specific human organs in a quantifiable manner is currently a focal point of intensive research and development. However, the long-standing inadequacies inherent to the use of conventional techniques for predicting human tissue behavior encourage the development of more complex and integrative approaches. Body-on-a-chip systems utilize function-based cell models that accurately capture and predict multi organ complexity within correctly scaled and physiologically relevant platforms. We demonstrate a functional human 4-organ system under continuous flow conditions in a serum-free defined medium utilizing a pumpless platform for 28 days. This system, with extended functional viability, incorporated human primary liver cells to produce a more accurate human body-on-a-chip than cell lines. Computer simulations of the platform established flow rates and resultant shear stress within accepted ranges. Viability of the system was demonstrated for 28 days as well as functional activity of cardiac, muscle, neuronal and liver modules. The presented phenotypic culture model exhibits function over 28 days, representing the next generation of *in vitro* systems for repeat-dose systemic toxicity screening. Further steps involve the integration of a skin module, mimicking a cosmetic application via topical exposure.

2478 Evaluation of Chemically-Induced Choline Deficiency as the Mode of Action (MOA) for Diethanolamine-Induced Mouse Liver Tumors

B. J. Hughes[4], J. Reichard[5], L. Haber[5], J. Klaunig[3], P. Fenner-Crisp[2] and J. Busch[1]. *[1]American Chemistry Council, Washington, DC; [2]Independent Consultant, North Garden, VA; [3]Dept of Environmental Health, Indiana University, Zionsville, IN; [4]Toxicology, Environmental, Research, and Consulting, The Dow Chemical Company, Midland, MI and [5]University of Cincinnati, TERA Center, Cincinnati, OH.* Sponsor: B. Hughes.

Diethanolamine (DEA) is used as an intermediate in the production of herbicides and personal care products and in natural gas treatment applications. Dermal exposure to DEA has been shown to increase liver tumors in B6C3F1 mice. DEA is non-genotoxic (not DNA reactive) in both *in vitro* (with and without metabolic activation) and *in vivo* assays. In our analysis, we reviewed the scientific literature related to DEA induced mouse liver tumors. We also evaluated the hypothesis that DEA induces mouse liver tumors via choline deficiency (CD), a non-mutagenic mode of action (MOA). Choline is an essential nutrient for maintaining methylation status, phospholipid biosynthesis, and cell signaling. In rodents, CD leads to tumor formation in the liver; CD is an important tool in rat liver tumor models used in dietary research. The modified Bradford Hill criteria were used to evaluate each key event (KE). The postulated KEs, supported by published data, include 1) cellular choline deficiency, 2) altered expression of key regulatory genes, and 3) selective growth of preneoplastic hepatocytes. We concluded that the data do not support a mutagenic mode of action (MOA) for DEA liver carcinogenicity. The available data support choline deficiency as the most plausible carcinogenic MOA for mouse liver tumor induction by DEA, exhibiting a threshold dose-response. Furthermore, choline deficiency as a MOA for liver tumors is of limited relevance to humans. We would propose additional research to fully confirm this hypothesis.

2479 Assessment of the Impact of Different Anesthesia Methods on Clinical Pathology Parameters and Organ Weights on Rodent Studies

M. Chen, H. Pei, F. Yu, S. McPherson, W. Lv and Z. Chengen. *Toxicology, Wuxi AppTec, Suzhou, China.*

Pentobarbital and isoflurane are both acceptable and established methods of anesthesia employed in toxicology studies. However, these are variables that need to be assessed, as they could impact parameters that are evaluated during the course of a toxicology study, i.e. clinical pathology and organ weights. In order to evaluate two anesthesia methods (pentobarbital and isoflurane), one hundred and twenty rats (Wistar Han [Crl:WI(Han)] Rat, SPF (Specific Pathogen-Free supplied from Charles River Laboratories USA) were euthanized following isoflurane anesthesia. At termination, blood samples for hematology, coagulation, and clinical chemistry analysis were obtained from the abdominal aorta of the rats prior to necropsy. Animals were euthanized via exsanguinations following blood collection. Selected organs were weighed. Animals were fasted overnight but had access to water prior to blood collection and necropsy. Paired organs were weighed together. Relative (to brain weight and to body weight) organ weights were calculated based on brain weight and terminal body weight. The obtained results were compared with historical background data which the pentobarbital anesthesia method was employed. Analysis of the data showed that of the 120 rats blood samples were successfully obtained from 97% of the animals. Comparison of the data obtained to the historical background data did not reveal significant differences for majority of the parameters except two parameters (White Blood cell count (WBC)and Glucose GLU)), which are explainable due to the different bleeding method and procedure. Organ weight individual data was compared to background ranges and there were no significant differences and all values were within the test site historical ranges. In conclusion both anesthesia methods are considered acceptable for rodent studies; and there were no significant changes on clinical pathology parameters or organ weights. However, it is recommended that the method of anesthesia be kept consistent throughout a toxicology program.

2480 Cinnamaldehyde, an Electronic Cigarette Flavoring, Adversely Effects Human Pulmonary Fibroblasts and Embryonic Stem Cells

R. Z. Behar, Y. Wang and P. Talbot. *University of California, Riverside, Riverside, CA.*

Chemicals used for flavoring in electronic cigarettes (EC) are a growing concern as many are generally regarded as safe, but the effects of heating and inhaling are unknown. Cinnamaldehyde (CAD), a chemical used to flavor cinnamon products, is of interest as it is present in EC refill fluids in concentrations that are cytotoxic to human cells. The purpose of this study was to evaluate the toxicity of CAD and determine its mechanism of action on human pulmonary fibroblasts (hPF) and H9 human embryonic stem cells (hESC) which model the adult lung and prenatal development, respectively. hESC are more sensitive than hPF after 48 hours of exposure to CAD in the MTT assay. However, using a reversal assay, hPF were more sensitive after a short exposure to CAD and were unable to recover after 3 hours of treatment, whereas, hESC can be exposed to CAD for more than 8 hours and still revert back to the morphology of control cells. Using immunocytochemistry, CAD caused tubulin depolymerization in hPF, and in both cell types, fluorescently labeled nuclei became brighter and condensed as the concentration of CAD increased, a phenomenon associated with cells that will undergo apoptosis. Concentrations of CAD that were not cytotoxic in the MTT assay produced genotoxic effects in the comet assay. Moreover, in hPF treated with CAD, a decrease in the levels of pro-inflammatory cytokines, IL-6 and IL-8, as well as a decrease in ADAMTS1, a protein associated with acute inflammation. Two additional proteins, HSP70 and CITED-2, were increased in treated cells. Both proteins are involved in cell stress related processes and have a role in apoptosis regulation. These data collectively indicate that CAD is cytotoxic and genotoxic to hESC and hPF, while also having anti-inflammatory effects on hPF. Inhaling heated refill fluids that contain CAD may alter cell health and decrease an immune response in the respiratory system, thereby increasing the likelihood of infection within the lungs.

2481 Polymorphisms in *FcgR3A* and *TAP1* Genes Affect Antibody-Dependent Cell-Mediated Cytotoxicity Activity in Cynomolgus Monkeys

J. C. Sanford, H. Wu, J. Harney and K. Adkins. *Drug Safety R & D, Investigative Toxicology, Pfizer Inc., Groton, CT.*

The cynomolgus monkey is commonly used to evaluate toxicity during drug development. However, genetic variability in this species and its relationship to human variability remains largely unexplored, particularly in the context of drug response. Human polymorphism (158V/F) in the Fc gamma receptor IIIA (FcgR3A) influences rituximab response via altered antibody-dependent cell-mediated cytotoxicity (ADCC) activity. Further, loss of function variants in human antigen peptide transporter 1/2 (TAP1/2) may decrease natural killer (NK) cell ADCC activity. The objective of this study is to evaluate whether genetic variation in cynomolgus monkey FcgR3A and TAP1 genes impact ADCC activity. ADCC was assessed using peripheral blood mononuclear cells (PBMCs) from cynomolgus monkeys treated with trastuzumab in the presence of NCI-N87 cells. Previously identified variants in FcgR3A and TAP1 were genotyped and association with trastuzamab-mediated ADCC activity was determined. FcgR3A 1134 A>C (exonic S41R) was significantly associated with decreased ADCC activity at trastuzumab concentrations ≥ 0.01 μM ($p<0.05$, 2-tailed t-test). FcgR3A 5027 A>G (intronic) and TAP1 1A>G (start codon loss) were also associated with decreased ADCC activity at 0.0001 to 0.001 μM ($p<0.05$, linear regression). Haplotype analysis demonstrated significant association of the SNP-SNP pairs FcgR3A S41R,TAP1 1A>G and FcgR3A 5027 A>G,TAP1 1A>G with decreased cytotoxicity at 0.0001 to 0.1 μM ($p<0.05$, linear regression). Mechanisms underlying altered ADCC activity were investigated by measuring FcgR3A IgG binding capacity and expression of FcgR3A on NK cells, though no associations were observed. In summary, haplotypes including FcgR3A S41R or 5027 A>G and TAP1 1A>G were significantly associated with decreases in trastuzamab-mediated ADCC activity in cynomolgus monkey PBMCs. This suggests genetic variation in cynomolgus monkeys is reflective of known human genetic variation and may potentially impact drug response to therapeutic antibodies.

2482 Evaluation of Glutamate Dehydrogenase as a Marker of Liver Injury in Human Patients with Evidence of Muscle Injury

S. Schomaker[2], R. Warner[3], K. Johnson[3], M. Binks[1], L. Fitz[1], S. Marraffino[1] and J. Aubrecht[2]. *[1]Pfizer Inc., Cambridge, MA; [2]Pfizer Inc., Groton, CT and [3]University of Michigan Medical School, Ann Arbor, MI.*

Alanine aminotransferase (ALT) is the most commonly used biomarker of liver injury in clinical practice. However, the leakage of ALT from muscle tissue limits its utility as a biomarker of liver injury in subjects with underlying muscle damage. The objective of this study was to assess the utility of glutamate dehydrogenase (GLDH) as a specific marker of liver injury in subjects with concurrent muscle injury. GLDH, ALT, aspartate aminotransferase (AST), and creatinine kinase (CK) were assessed in cohorts of human subjects including 127 healthy males, 35 boys with Duchenne muscular dystrophy (DMD), 218 subjects with varying degrees of muscle impairments and 8 patients with liver injury caused by acetaminophen (APAP) poisoning. All analytes were measured in serum on the Siemens ADVIA 2400 Automated Chemistry System using commercially available kits. GLDH was elevated in the 8 subjects with APAP overdose which were included in the analysis to demonstrate the ability of GLDH to detect liver injury. Samples collected from DMD boys showed elevations in levels of ALT (19x), AST (11x) as well as increased level of CK (107x). In contrast, the GLDH level in DMD boys did not differ from healthy adults and healthy boys. We also evaluated the correlation of levels of GLDH with CK, a widely used biomarker of muscle injury, in patients with a variety of muscle impairments. There was a poor correlation between GLDH and CK ($rs = 0.39$) confirming absence of GLDH release from damaged muscle. However, there was an excellent correlation between ALT and CK ($rs = 0.75$) indicating that the ALT originated from muscle in these subjects. An evaluation of biomarker time courses in 4 cases of rhabdomyolysis provided further evidence that GLDH is applicable for monitoring liver injury in subjects with concurrent muscle injury. These clinical studies demonstrated that, in contrast to ALT, GLDH serum levels are unaffected by concurrent muscle damage and can be utilized to monitor for liver injury in human patients with underlying muscle injury.

2483 Glutathione Depletion Circumvent Melphalan Resistance in Multiple Myeloma

W. Hassen[2,5], C. Gourzones[5], S. Lamure[5], O. Karmous[1,5], D. Hose[4], G. Cartron[3], B. Klein[1,5] and J. Moreaux[1,5]. *[1]Laboratory for Monitoring Innovative Therapies, Department of Biological Hematology, CHRU Montpellier, Montpellier, France; [2]Department of Cellular Physiology and Toxicology, High Institute of Biotechnology, Monastir, Tunisia; [3]Department of Clinical Haematology, CHU Montpellier, Montpellier, France; [4]Universitätsklinikum Heidelberg and Nationales Centrum für Tumorerkrankungen, Medizinische Klinik V, Heidelberg, Germany and [5]UPR1142, Institute of Human Genetics, Montpellier, France.*

Alkylating agents are widely used chemotherapeutics in the treatment of many cancers. Melphalan is the most commonly used chemotherapeutic drug against multiple myeloma (MM), a malignant plasma cell disease accounting for approximately 10% of haematological malignancies. However, despite 70% initial response rate, virtually all patients relapse due to the emergence of drug-resistance. In this work, we explored the mechanisms of Melphalan resistance in human myeloma cells in order to identify targets that might alleviate drug resistance and improve therapeutic outcomes. Using both Melphalan resistant and sensitive human myeloma cell line (HMCL), we investigated DNA repair 53BP1 foci and found no difference between sensitive and resistant cells inferring that Melphalan resistance is not attributed to improved DNA repair capacity. Next, we demonstrated that Melphalan treated sensitive cells suffered higher oxidative damage including increased protein carbonyls, intracellular Glutathione depletion, lipid peroxidation and up regulation of Nrf2 target genes. In addition, we demonstrated that Melphalan resistant cells carried higher levels of intracellular Glutathione and that Melphalan treatment does not lead to any detectable oxidative lesions. We hypothesised that different intracellular Glutathione level could explain Melphalan resistance. We next showed that treatment with a Glutathione inhibitor, BSO sensitizes cells to Melphalan treatment and circumvents resistance whereas treatment with Glutathione induces a remarkable increase in survival rate in HMCL and primary culture of MM cells. Melphalan appears to kill myeloma cells through induction of oxidative damage. The intracellular Glutathione level is determinant to Melphalan resistance in MM suggesting that the use of Glutathione inhibitors such as BSO, in combination with Melphalan could be an attractive approach to circumvent Melphalan resistance.

2484 Risk Factors of Drug-Induced Hepatotoxicity in Acute Paracetamol Overdose Among Patients Admitted at Philippine General Hospital from January 2010 to December 2014

E. J. N. Perez[1,2] and C. P. C. Dioquino[1]. *[1]National Poison Management and Control Center, Philippine General Hospital, Manila, Philippines and [2]Poison Treatment and Control Center, Southern Philippines Medical Center, Davao City, Philippines.*

Background: Paracetamol overdose is the most apparent cause of acute liver failure in United Kingdom and United States with high percentage of fulminant liver failure requiring liver transplantation. Among Asian countries, studies showed low rates of paracetamol induced hepatotoxicity with details on its predictors. No reports of acute liver failure and liver transplantation. Objectives: To determine the incidence of acute paracetamol overdose in Philippine General Hospital from January 2010-December 2014, describe the characteristics in terms of demographic, clinical and laboratory data of patients with acute paracetamol overdose, determine factors that predispose patients with acute paracetamol overdose to develop paracetamol induced hepatotoxicity and to determine the morbidity and mortality of patients with acute paracetamol overdose during their course of treatment. Method: An approval from the University of the Philippines, Manila Research Ethics Board panel was obtained. The study examined and reviewed the medical charts and poison database of all patients with acute paracetamol overdose. The data gathered were expressed as frequency, mean, range and percentages. The identified common factors were explored using logistic regression. Evident limitation of the study was its small sample size because most of the cases had co-ingestion of other medications. Only 30 cases were included and analyzed. Results: Results showed an increase in the incidence of acute paracetamol overdose cases. 37% (n=11) developed paracetamol induced hepatotoxicity. The mean amount ingested was 15 grams There were five factors identified to be common among patients who developed paracetamol induced hepatotoxicity and these were older age, onset of symptoms of more than 4 hours after ingestion, ingestion of a toxic dose, category of Rumack-Matthew propable risk for hepatotoxicity and the presence of leukocytosis. Only a toxic dose ingested (OR4.7, 95% CI 2.76-6.21) and the presence of leukocytosis (OR 7.14, 95% CI 2.7-9.12) had significant association with the development of paracetamol induced hepatotoxicity. None of the patients developed acute liver failure and all patients were discharged well with mean hospital stay of 3 days

2485 Changes in the Blood and Some Serum Chemistry Parameters of Wistar Rats Exposed to Methanolic Extract of the Root Bark of *Afromosia laxiflora*

G. M. Oladele. *Department of Veterinary Pharmacology and Toxicology, University of Abuja, Abuja, Nigeria, Abuja, Nigeria.*

Afromosia laxiflora Benth is a medicinal plant that has been used for the treatment of various ailments of human and animals overtime. Although medicinal plants are widely used and assumed to be safe, however, they can be toxic thereby posing danger to the users hence the need to carry out toxicity study. Afromosia laxiflora has been found to be useful in the treatment of pain, diarrhoea, dysentery, gout and also as antidepressant and anticonvulsant. Methanolic extract of the root bark of this plant was administered orally to 3 groups of rats containing 6 rats in each group at 250, 500 and 1000 mg/kg doses respectively while the control group was administered with distilled water orally. The administrations were done for 21 days and blood was thereafter obtained from the anaesthetized rats through cardiac puncture. The results of the blood and serum analyses showed a decrease in red blood cell count, haemoglobin concentration and an increase in total white blood cell and lymphocytes. The total protein slightly decreased but alanine aminotransferase and aspartate aminotransferase significantly ($p<0.05$) increased. It was discovered that the extract could be toxic to the liver as the aminotransferases significantly increased and increase in white blood cell could possibly help the immunity of the rats.

2486 Human Corneal Wound Healing Is Enhanced in an *Ex Vivo* Front of the Eye Model

A. E. Vickers[2], J. E. Herrmann[1] and R. L. Fisher[3]. *[1]Allergan, Irvine, CA; [2]Human Translational Models, Irvine, CA and [3]Vitron Inc, Tucson, AZ.*

Corneal injury triggers an inflammatory reaction and the activation of cells to release mediators to induce healing and reepithelialization. Several types of compounds are demonstrating an acceleration in the wound healing process: 1) the lipid mediator lipoxin (LXA4) acts as a second messenger for EGF to promote proliferation and repair; 2) the opioid antagonist naltrexone (NTX) promotes cell proliferation and architectural integrity; and 3) the prostacyclin analog cicaprost (CP) modulates the inflammatory response via activation of the prostanoid IP receptor. The effect of these compounds on human corneal wound healing was investigated in a human *ex vivo* front of the eye model. Human eyes were procured with donor consent through Midwest Eye-Banks (Ann Arbor, MI) and The Lions Eye Institute for Transplant and Research (Tampa, FL). The eyes were surgically wounded, 8 mm diameter anterior keratectomy (AK), by scraping the epithelium and part of the stroma. The anterior segment was then dissected and the eye cultured in complete medium on a rotating platform to maintain tissue differentiation for several days. Each drug was administered to an eye pair topically (1 drop at 0 and 9 hr): LX 100 nM, NTX 1 μM, and CP 0.1%. The initial wound and progression of wound closure (0, 18 hr) was visualized with 0.015% fluorescein and the area quantified and compared to the AK vehicle matched eye. Each drug enhanced wound healing. Variation in closure rate was evident across human eye pairs and for compounds. The rate of wound closure ranged from 0.5-1.7 mm2/hr in the drug treated eyes compared to 0.3-1.4 mm2/hr in the vehicle treated eyes, representing a percent increase in closure rate ranging from 0-257% for LXA4 (n=3), 10% for NTX (n=1), and 18-117% for CP (n=3).

2487 Metformin Scavenges Methylglyoxal to Form an Imidazolinone Metabolite in T2D Patients: A Potential Therapeutic to Alleviate T2D Complications

T. L. Hargraves. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.* Sponsor: S. Lau.

Reactive dicarbonyls, such as methylglyoxal (MG), are elevated in type-2 diabetes mellitus (T2DM) patients. These endogenous electrophiles covalently modify proteins, which may contribute to diabetic complications. The T2DM first-line therapy metformin (MF) significantly reduces diabetic endpoints and mortality more effectively than other anti-hy-

perglycemic agents. We examined whether MF directly scavenges MG as an alternative mechanism of drug efficacy, in addition to its antigluconeogenesis mechanism. We synthesized and characterized a MF/MG imidazolinone product (IMZ) by 13C, 1H and HMBC NMR, LC-MS/MS and X-ray diffraction. We measured MF and the IMZ product in human urine using an LC/MS multiple reaction monitoring (MRM) method. The IMZ was detected in >90 MF-treated T2DM patients with increased levels of MF directly correlating with elevations in IMZ. Urinary MF was detected in the range of 0.17 μM -23.0 mM. Simultaneously the IMZ concentration was measured between 18.8 nM and 4.3 μM. This level of IMZ production is therapeutically significant as reported plasma concentrations of MG ranges from 40 nM to 4.5 μM. To investigate the significance of IMZ in the overall scheme of metformin drug efficacy we examined the mechanism of IMZ in modulation of the AMPK and MAPK signaling pathways via western blot. In HepG2 cells, MF increased pAMPK, pACC, and pRaptor while IMZ increased pAMPK and pRaptor, however, decreased pACC levels. These results indicate that IMZ can potentiate MF effects in the insulin-dependent AMPK pathways. The decrease in pACC may represent an interesting regulatory pathway in fatty acid biosynthesis that is of importance in other diseases. In summary, the concordance of IMZ concentration provides evidence that metformin is scavenging MG thereby reducing detrimental protein modifications, which may play a role in the reduction of diabetic complications. (R24DK0090958, T32ES007091, P30ES006694).

2488 Predictivity of Animal Models for Hepatotoxicity: Two Classes of Drugs

W. B. Mattes. *Division of Systems Biology, US FDA NCTR, Jefferson, AZ.*

The seminal paper by Olson and colleagues in 2000 explored the question of the "concordance of the toxicity of pharmaceuticals in humans and in animals". Through a consortium approach this study explored the preclinical data for 150 compounds with 221 human toxicity events. The results have been considered both as supportive and unsupportive for the predictivity of animal models for human safety. To revisit the predictivity of animal studies we made use of a database (PharmaPendium) that provides tabular access to regulatory submission data, and focused on drugs with reports of hepatotoxicity in clinical trials and then examined the non-clinical data in the FDA submissions for two classes of approved small molecule drugs: antivirals (AVs) and tyrosine kinase inhibitors (TKIs). For these two classes there was 2224 and 1056 human hepatotoxicity events, respectively, recorded in clinical trials. To assess predictivity, the doses for animal studies were first converted to a human equivalent dose (HED) using allometric scaling, and then normalized to a Therapeutic Dose Fraction (TFD) by dividing the HED by the daily therapeutic dose as found in the FDA label. Thus a finding in a non-clinical study at a TFD of 1 indicates that the non-clinical dose is equivalent to the clinical dose. Of the 38 AVs, with a total of 900 animal liver toxicities recorded, 31 had such toxicities at TFDs between 0.2 and 5 (i.e. within 5 fold of the clinical dose). Of the 24 TKis, with a total of 516 animal liver toxicities, 23 had such toxicities at TDFs between 0.2 and 5. These preliminary data suggest that while there may be specific mechanisms of human toxicity not replicated in animal models, overall, non-clinical animal models do indeed provide reliable signals of human toxicity, at least in the case of the hepatotoxicity of these two classes of drugs.

2489 Regulation of Secreted miRNAs and P450 Isoforms in Acetaminophen Metabolism in HepaRG Cells and Acetaminophen Overdose in Children

P. S. Gill[2], S. Bhattacharyya[2], S. McCullough[1], P. Mishra[3], C. Luo[1], L. Letzig[1] and L. James[1]. [1]*Pediatrics, University of Arkansas for Medical Sciences, ACHRI, Little Rock, AR;* [2]*Pediatrics, University of Arkansas for Medical Sciences, ACHRI, Little Rock, AR and* [3]*Small Molecule Drug Discovery and Biochemical and Cellular Pharmacology, Genentech, San Francisco, CA.*

Acetaminophen (APAP) is an important cause of acute liver injury. In children, APAP overdose accounts for 13% of cases of acute liver failure, and the etiology of liver injury in up to 50% of cases is unknown. This study examined miR-122-5p, as well as microRNAs (miR-378a-5p, -27b-3p and -320a) that regulate the Cytochrome P-450 isoforms (CYP2E1, CYP3A4 and CYP1A2), involved in APAP metabolism in HepaRG cells and in serum samples obtained from children with APAP overdose. miRNA expression was compared to ALT, LDH, and APAP protein adducts. HepaRG cells treated with 20 mM APAP had elevation of APAP protein adducts at 1 h and elevation of ALT and LDH at 12 and 24 h, respectively. CYP2E1, CYP1A2 and CYP3A4 mRNA expression levels were suppressed with 20 mM APAP treatment ($p<0.05$), while expression levels of secreted miRNA showed significant induction of miR-122-5p, -125b-5p, -378a-5p, and -27b-3p at 6 h, miR-125b-5p at 12 h, and miR-27b-3p at 24 h. Similar findings were observed in the serum samples of children with APAP toxicity (N=12, median age 15.79 y; median ALT levels of 1266.5 IU/L, median APAP protein adduct levels of 1.15 nmol/L) when compared to control subjects (N=15, median age: 7.42 y; median ALT of 16.0 IU/L; median APAP protein adducts 0.0053 nmol/L). A 140 fold increase in miR-122-5p was observed in children with APAP toxicity, compared to healthy controls ($p<0.05$). miR-378a-5p, a regulator of CYP2E1, was increased by 12.6 fold in APAP overdose subjects compared to healthy controls, while miRs -125b-5p, -320a and -27b-3p were increased by 8.4, 2.3 and 4.4 fold, respectively, compared to controls. In children with APAP overdose, APAP protein adducts correlated with miR-27b-3p (R=0.68), whereas ALT correlated with miR-122-5p (R=0.89) and miR-378a-5p (R=0.82) at $p < 0.05$. Overall, expression of miRNAs that regulate CYP2E1, CYP3A4 and CYP1A2 metabolism are increased in serum samples of children with APAP toxicity, in agreement with *in vitro* data using HepaRG cells exposed to APAP. miR-122 levels in children with APAP toxicity are comparable to levels previously observed in adults with APAP toxicity.

2490 Next-Generation Sequencing Detects Differences in the Airway Microbiome After Smoke-Induced Inhalation Injury by Injury Type and Associated Bacterial Species

D. M. Walsh[1], S. D. McCullough[2], S. Yourstone[1], J. S. Kahle[2], S. W. Jones[1], C. D. Jones[1], I. Jaspers[1] and D. Diaz-Sanchez[2]. [1]*University of North Carolina at Chapel Hill, Chapel Hill, NC and* [2]*US EPA, Research Triangle Park, NC.*

Over 3 billion people who rely on burning fuels for heat and cooking are at increased risk of respiratory infections and chronic diseases due to smoke inhalation. Smoke-induced inhalation injury contributes significantly to morbidity and mortality by damaging the airways and increasing susceptibility to infection. Respiratory infections may lead to pneumonia if the infecting organism is not identified and treated early. Identification of bacterial infections can be challenging due to the complex communities of species that colonize the airways and our inability to culture the majority of colonizing bacterial species. To address this limitation, we used next-generation sequencing methods to identify the microbiome present in the airways within the first 72 hours following burn and inhalation injury. Specifically, DNA was extracted from bronchial washings of 48 patients hospitalized for burn and inhalation injury at the North Carolina Jaycee Burn Center. Burn victims with inhalation injury provide a model in which to study the impact of high-dose smoke exposure on bacterial colonization of the airways. We hypothesize that inhalation injury inhibits airway defense mechanisms, allowing infection by bacteria that can lead to pneumonia. The bacterial genus Streptococcus dominated the overall bacterial population, comprising 24.7% of the community. The anaerobic bacteria Prevotella was significantly enriched in patients with acute lung injury (ALI), where it accounted for 75% of total Prevotella detected among the patients. Poor oxygenation by the lungs, established by conditions such as those consistent with ALI, might create anaerobic microenvironments within the airways that encourage colonization by Prevotella. This work identifies the airway microbiome after smoke-induced inhalation injury and specific changes induced by type of injury. Outgrowth of minor species, like Prevotella, has been significantly associated with disease in other body locations, indicating the significance of their contribution to microbiome dynamics. Early detection of bacterial interactions in the airways following smoke exposure may identify risk factors for infection that can be used to improve health outcomes. This work does not reflect EPA policy.

2491 Inflammatory Mediator Generation in Surviving and Non-Surviving Acetaminophen Overdose Patients

B. Woolbright[2], M. McGill[2], M. Sharpe[2], S. Curry[1] and H. Jaeschke[2]. [1]*Banner Good Samaritan Medical Center, Phoenix, AZ and* [2]*Kansas University Medical Center, Kansas City, KS.*

Acetaminophen (APAP) overdose is a leading cause of acute liver failure in the US. In human overdose patients, metabolic activation of acetaminophen leads to mitochondrial dysfunction, hepatic inflammation, and parenchymal cell death. Considerable debate continues to occur over whether inflammation exacerbates injury after an overdose, or helps repair the damaged liver through clearance of necrotic debris. Currently, there is limited information on the time course of cytokine formation and serum complement activation in human patients, especially in surviving (S) and non-surviving (NS) populations. This study hypothesized NS patients would accumulate a greater degree of plasma cytokine levels, and have more severe complement depletion after APAP overdose.

Circulating plasma levels of IL-1ß, TNF-α, IL-6, IL-8, IL-10, MCP-1, G-CSF, and GRO were measured by multi-plex ELISA and complement C3 was measured by traditional ELISA in healthy volunteers (n=8), patients with APAP overdose but no increase in liver enzymes (n=10), and S (n=13 at initial time point) and NS (n=8 at initial time point) APAP overdose patients with liver injury, for the first seven days after study admission. ALT levels were similar between S and NS groups at peak injury and throughout the time course, although bilirubin was significantly greater in the NS group. Plasma levels of pro-inflammatory cytokines such as IL-1ß, GRO, and TNF-α did not rise above control levels. In contrast, S and NS patients had significant elevations in G-CSF, IL-6, IL-8, and anti-inflammatory cytokines such as IL-10 and MCP-1. Moreover, levels of IL-6, IL-10 and MCP-1 were significantly elevated in NS patients compared to S patients at multiple time points. MCP-1 levels rose to the greatest degree in S patients (6,134 ± 1,134pg/mL) and NS patients (7,074 ± 1,105pg/mL); whereas IL-1ß accumulated the least in S patients (81.4 ± 64.2pg/mL) and NS patients (39.2 ± 32.6pg/mL). Plasma C3 levels were depleted at the same rate in S and NS patients, but recovered more quickly in S patients, likely indicative of hepatic regeneration and restored function. In conclusion, these data support the hypothesis NS patients become refractory to anti-inflammatory cytokine signals which fail to resolve injury or stimulate regeneration. Additionally, specific cytokines may have predictive value for acute liver failure.

PS 2492 PXR and CAR Nuclear Receptors Play a Key Role in Maintaining Drug Metabolizing Enzymes Levels in Untreated and Treated Rats

K. P. Forbes and X. Cui. *Research and Development, Horizon Discovery, St. Louis, MO.* Sponsor: T. Brayman.

The nuclear pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are transcription factors involved in the regulation of drug metabolizing enzymes and transporters in response to xenobiotics. Null and humanized mouse models for these receptors have been generated, characterized (for review see ref 1), and been a very important part of the toxicity evaluation during the drug development process. Because of their larger size, rats are a preferred ADME/T model system over mice. We created PXR and CAR knockout rats in Sprague Dawley (SD) background using ZFN technology (2). In addition, we cross-bred these knockouts to generate the double knockout for PXR and CAR (PXR/CAR). In this study, we treated 8-week old wild-type and null male SD rats with known rodent activators of PXR, pregnenolone-16α-carbonitrile (PCN) and CAR, 1,4-Bis-[2-(3,5-dichloropyridyloxy)]benzene, 3,3′,5,5′-tetrachloro-1,4-bis(pyridyloxy)benzene (TCPOBOP). RNA was extracted from liver tissue of treated animals and analyzed via qRT-PCR (SYBR-arrays and Taqman assays). PCN treated null-PXR and null-PXR/CAR rats loss the activation of the Cyp3a4 family members (Cyp3a18, 3a23/3a1, 3a2) and Cyp2b2 as compared to treated wild-type rats. Conversely, in PCN treated null-CAR rats, Cyp2b2 was activated and another Cyp3a4 family member (Cyp3a9) loss activation as compared to treated wild-type rats. Both PXR and CAR single knockouts and the double knockout rats, loss the activation of Cyp2b2 after treatment with TCPOBOP. In the null-PXR and PXR-CAR rats, we observed higher constitutive levels of Cyp2b2 and Cyp3a4 family members (Cyp3a18, 3a23/3a1, 3a2) as compared to wild-type rats. In the null-CAR rats, we observed lower and higher constitutive levels of Cyp2b2 and Cyp3a9 respectively. This suggests that PXR and CAR play key regulatory roles in maintaining constitutive levels of drug metabolizing genes. These models alone should be useful for studying metabolism of xenobiotic compounds and hepatotoxicity. In addition, these models are also critical components for the humanization of the cytochrome P450 pathways in the SD rat. 1. Scheer N, Wolf CR, (2013) Xenobiotic receptor humanized mice and their utility Drug Metabolism Reviews 45(1): 110-121. 2. Geurts et al., (2009) Knockout rats via embryo microinjection of zinc-finger nucleases. Science 325(5939):433

PS 2493 Co-Culture of Human Hepatic Stellate Cells and Hepatocytes for Physiological Modeling of Liver Fibrosis

M. Kumar, R. M. Witek, B. Khaniya, J. A. Bonzo, M. J. Powers and R. P. Witek. *Cell Biology, Thermo Fisher Scientific, Frederick, MD.*

Human hepatic stellate cells (HSC) are vitamin A storing cells in the perisinusoidal space of Disse located between sinusoidal endothelium and hepatocytes. In normal liver, stellate cells store vitamin A in the form of retinol droplets and are considered quiescent (q-HSC). Following liver damage, hepatic stellate cells activate to a phenotype characterized by increased proliferation, motility, contractility, and synthesis of extracellular matrix components. This activation is regulated by cytokines, chemokines, and growth factors. To understand the activation process of q-HSC and to facilitate the understanding of their effect on hepatocyte function, we used co-culture of isolated quiescent hepatic stellate cells with primary hepatocytes. Quiescence of isolated HSC's was established using vitamin A, presence of GFAP and absence of α-SMA. As expected, culturing these cells activated them to myofibroblasts shown by α-SMA staining. Activated HSCs were also positive for Vimentin and CD271 indicating their mesenchymal origin. Expression of TGF-β and PDGFr-β was used to determine their functionality. In order to understand the role of various factors on the activation, q-HSCs were maintained in culture until activation. Cytokine, chemokine and growth factors production was monitored by Luminex multiplex panels and qRT-PCR was used to evaluate gene expression. When co-cultured with hepatocytes, either directly or in a trans-well culture, HSCs stabilized the culture of primary human hepatocytes or elicited response observed during liver fibrosis. In conclusion, we have generated primary human quiescent and activated hepatic stellate cells with high purity and function. Our data indicates involvement of HSC produced cytokines and chemokines in function of hepatocytes with implications in modeling fibrosis for drug metabolism and toxicity testing.

PS 2494 Epigenetic Mechanism for Chronic Oxidative Stress-Induced Resistance TT Doxorubicin Cytotoxicity in Renal Carcinoma Cells

L. Ponnusamy, P. K. S. Mahalingaiah and K. P. Singh. *Environmental Toxicology, The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX.*

Renal cell carcinoma (RCC) is the most common form of kidney cancer in human. Both inherent and acquired resistance to chemotherapy are major challenges in kidney cancer treatment. Chemoresistance arise through a number of factors. Although plenty of evidence support role of ROS in kidney cancer development, effect of chronic oxidative stress on chemotherapy response is unclear. Therefore, objective of this study was to evaluate the impact of chronic exposure to elevated levels of oxidative-stress on doxorubicin-cytotoxicity in human kidney cancer cells. To achieve this objective, Caki-1 human RCC cells adapted to chronic H2O2-induced oxidative stress were used for evaluating sensitivity to doxorubicin. Doxorubicin-cytotoxicity was measured by MTT assay and confirmed by gene expression and cell cycle analysis. Soft-agar and wound healing assays were performed to determine effect of oxidative stress on anchorage-independent growth and cell migration potential. To delineate molecular mechanism, changes in epigenetic regulatory genes and epigenetic modifications were also evaluated. Results of MTT, cell cycle, and soft-agar assay revealed that doxorubicin-cytotoxicity was significantly less in chronic oxidative stress-adapted Caki-1 cells when compared to unexposed control. Changes in expression of genes involved in drug transport, survival, and DNA repair-dependent apoptosis further confirmed increased resistance to doxorubicin in oxidative stress-exposed cells. Down-regulation of MSH2, a mismatch repair (MMR) gene suggests that loss of MMR-dependent apoptosis a possible mechanism for increased resistance to doxorubicin. DNA demethylation by 5-aza-2′deoxycytidine treatment significantly restored expression of MSH2 gene and resensitized chronic ROS-adapted Caki-1 cells to doxorubicin. Moreover, Pyrosequencing analysis further confirmed DNA hypermethylation of MSH2 promoter indicating silencing of MSH2 through DNA hypermethylation as a potential mechanism for chronic oxidative stress-induced resistance to doxorubicin. In summary, this study for the first time provides direct evidence for role of oxidative stress in chemoresistance in RCC. Findings of this study also suggest that epigenetic therapy may be a useful strategy in re-sensitizing chemotherapeutic resistant RCC cells

PS 2495 Development of a Multiplatform Assay Using High Content Screening and Microelectrode Array to Assess Cardio, Neuro, and Hepato-Toxicity in a Group of Antipsychotic Drugs

C. J. Strock, J. A. Bradley, S. Qin, K. Gareau and J. Gilbert. *Cyprotex US, LLC, Watertown, MA.*

Antipsychotics are a class of drugs used to treat psychoses such as bipolar disorder and schizophrenia. These drugs are generally divided into two categories; first generation antipsychotics (typical) and second generation antipsychotics (atypical). Both generations antagonize dopamine type 2 (D2) receptors, but the second generation also blocks serotonin 5-HT2A receptors. The first generation antipsychotics were introduced in the 1950s, and although proven to be effective treatments, are also associated with significant side effects. The second generation of antipsychotics emerged in the 1980s and were developed to minimize some of the side effects but are not without issues of their own. Among the side effects that have emerged for drugs from both generations are QT interval prolongation, seizures or lowering of the seizure threshold, and in some cases, hepatotoxicity. De-risking potential new drug entities *in vitro* is an important step in the drug discovery process,

particularly for classes of drugs that historically have significant side effects. Here we tested at a group of antipsychotics from both categories to assess the potential for cardiotoxicity, neurotoxicity and hepatotoxicity. These drugs were tested in three whole cell assays utilizing both microelectrode array (MEA) technology to detect the potential for sub cytotoxic electrophysiological liabilities, and a high-content screening assay using two different cells types to detect the potential for hepatic liabilities. Results from the MEA assays confirmed cardiac liabilities associated with hERG inhibition as well as neuroactive liabilities. The HCS hepatotoxicity assay identified liabilities associated with liver toxicity. Results indicate a good correlation with the data generated from these assays when compared to known *in vivo* liabilities and side effects from these drugs. Therefore, this suite of assays is a viable drug discovery screening tool for the early detection of potential toxic liabilities associated with cardiotoxicity, neurotoxicity and hepatotoxicity.

PS 2496 Suramin Protects from Cisplatin-Induced Acute Kidney Injury

T. V. Dupre[3], M. A. Doll[3], C. N. Sharp[3], P. P. Shah[3], A. Kiefer[3], M. T. Scherzer[3], K. Saurabh[3], D. Saforo[3], D. Siow[3], L. Casson[3], G. E. Arteel[3], A. B. Jenson[3], J. Megyesi[2], R. G. Schnellmann[1], L. J. Beverly[3] and L. J. Siskind[3]. *[1]Dept. of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Louisville, KY; [2]Dept. of Internal Medicine, University of Arkansas for Medical Sciences and Central Arkansas, Little Rock, AR and [3]Dept. of Medicine, University of Louisville, Louisville, KY; [4]Dept. of Pharmacology/Toxicology, University of Louisville, Louisville, KY; [5]James Graham Brown Cancer Center, University of Louisville, Louisville, KY*.

Cisplatin, a commonly used cancer chemotherapeutic, has a dose limiting side effect of nephrotoxicity. Approximately 30% of patients administered cisplatin suffer from kidney injury and there is currently no treatment for cisplatin-induced kidney injury other than palliative care. Suramin, FDA-approved for the treatment of trypanosomiasis, improves kidney function following various forms of kidney injury in rodent models. We hypothesized that suramin would attenuate cisplatin-induced kidney injury. Suramin treatment prior to cisplatin administration reduced cisplatin-induced decreases in kidney function and injury. Furthermore, suramin attenuated cisplatin-induced expression of inflammatory cytokines and chemokines, endoplasmic reticulum stress, and apoptosis in the kidney cortex. Treatment of mice with suramin 24 hours after cisplatin improved kidney function, suggesting that the mechanism of protection is neither via inhibiting tubular cisplatin uptake nor its metabolism to nephrotoxic species. If suramin is to be utilized in the context of cancer, then suramin cannot prevent cisplatin-induced cytotoxicity within tumors. Mice harboring lung adenocarcinomas were pretreated with suramin and then administered cisplatin. Lung histology and markers of kidney function indicated that suramin protected mice from cisplatin-induced acute kidney injury, but did not inhibit cisplatin induced cytotoxic effects within the tumor. These results provide evidence that suramin has great potential as a renoprotective agent for the treatment/prevention of cisplatin-induced acute kidney injury.

S 2497 New Mechanistic Insights into How the Immune System Drives Hepatic Adverse Drug Reactions

R. Roth[1] and B. van de Water[2]. *[1]Michigan State Univ., East Lansing, MI and [2]University of Leiden, Leiden, Netherlands.*

Idiosyncratic drug-induced liver injury (IDILI) remains a public health problem and a major reason for curtailing the use of otherwise efficacious drugs. Why some patients are uniquely sensitive to IDILI and the mechanisms by which the death of hepatocytes occurs during these reactions remain incompletely understood. Studies in humans as well as in animal and *in vitro* models have begun to provide answers to these questions. A major working hypothesis for IDILI is the involvement of the immune system in its onset. Over the past years, groundbreaking progress has been made to decipher drug-immune interactions in IDILI. *In vivo* models and immunological approaches have yielded insight into mechanisms by which both the innate and adaptive immune system contribute to IDILI liabilities. In addition, integration of transcriptomics and systematic RNA-interference approaches allowed the deciphering of underlying molecular mechanisms by which liver cells are susceptible to injurious cytokines. For several drugs that cause IDILI in human patients, the reactions appear to involve activation of an adaptive immune response, and specific HLA polymorphisms are risk factors. The first presentation will summarize results of a novel, murine model in which an HLA polymorphism resulted in sensitivity to flucloxacillin-induced liver injury that was associated with proliferation of CD8+ T-cells, hepatic leukocyte infiltration and secretion of interferon-gamma (IFNg), and other factors. Liabilities for IDILI likely involve specific cellular perturbation due to patient-specific disease states. Given the high incidence of viral hepatitis infection, specific viral infections are likely contributors to IDILI. The second presentation will focus on a murine model of ximelagatran-induced liver injury, in which livers are sensitized to damage by combined viral infection and tumor necrosis factor alpha (TNF). Interestingly, many IDILI-associated drugs can sensitize hepatocytes to cytokine-mediated cell killing. This is exemplified by the synergistic liver cell killing mediated by exposure to nonsteroidal, anti-inflammatory drugs (NSAIDs) and TNF and/or IFNg. Recent studies have begun to unravel mechanisms by which this synergistic drug-cytokine interaction causes cell death. For example, exposure to diclofenac causes endoplasmic reticular stress that renders hepatocytes susceptible to killing by TNF. Moreover, diclofenac modulates TNF-dependent activation of NFkB signaling. State-of-the-art RNA-interference approaches in combination with live cell imaging have contributed to the deciphering of molecular determinants that drive the drug-mediated sensitization to TNF. Since liver cell injury also causes the activation of stress kinases, there is a contribution of specific, drug-induced kinase signaling perturbations to the onset of liver cell death caused by TNF coexposure. The role of MAPKs such as JNK and ERK in the cytotoxic, synergistic interaction of drugs with TNF and IFNg will be discussed. Together, the presentations will convey a spectrum of factors that contribute to the mechanistic understanding of drug-immune interactions that contribute to IDILI. Integration of these novel insights into safety assessment strategies will likely reduce the late-stage attrition of drug candidates and bring safer drugs to the patient population.

S 2498 Immune Mechanisms of Idiosyncratic Liver Injury: Can We Predict the Unpredictable?

L. Lehman-McKeeman. *Bristol-Myers-Squibb Corp., Princeton, NJ.*

The prevalence of IDILI remains a serious issue in clinical safety and is a leading cause of compound attrition. Identification of this liability is hampered by the lack of detection until late clinical development or post-marketing. Additionally, the mechanistic basis for toxicity is not well understood. Overall, animal models have generally been of limited utility to date, and biomarkers for preclinical and clinical evaluation have been difficult to identify. However, emerging science indicates that an important mechanistic contributor in IDILI is the immune system. Moreover, although the pathogenic mechanisms underlying hepatocyte killing in IDILI reactions have been mysteries for a long time, recent studies have begun to provide meaningful insight into the hepatotoxic response. This introduction will define the breadth of the preclinical and clinical issues associated with IDILI and highlight the significance and potential applications of some of the emerging data that will be summarized in the presentations that follow.

S 2499 Activation of Drug-Specific Cytotoxic T-Cells in Patients with IDILI and Modeling the Reaction in Experimental Animals

D. Naisbitt. *University of Liverpool, Liverpool, United Kingdom.* Sponsor: R. Roth.

Idiosyncratic, drug-induced liver injury (IDILI) is an important clinical problem. Reactions are difficult to predict as mechanisms are not fully understood. Studies in humans have implicated specific HLA polymorphisms as risk factors for IDILI from flucloxacillin and other drugs (e.g., ticlopidine, lapatinib amoxicillin/clavulanate). For flucloxacillin we have characterized drug-responsive CD4+ and CD8+ T cells from IDILI patients and shown that naive CD8+ T cells from volunteers expressing the risk allele HLA-B*57:01 are activated *in vitro* with the drug. Recent studies have also detected drug-specific T-cells in patients with liver reactions to co-amoxicillin/clavulanate, isoniazid and ticlopidine, effectively defining an important role for the adaptive immune system in several forms of IDILI. The phenotypic and functional characteristics, HLA restriction and cross-reactivity profiles of these cells will be reviewed. Cloned drug-specific T-cells proliferate in a concentration-dependent manner and secrete IFN-γ and cytolytic molecules including perforin, FasL and granzyme B.There is now a need to develop novel cellular platforms to investigate the mechanisms of emerging drug safety issues where an HLA-risk allele has been identified. Thus, I will describe how it is now possible to use blood lymphocytes from HLA-genotyped healthy donors to (1) study immunological responses to drugs and drug metabolites and (2) determine whether T-cells kill primary hepatocytes. The discoveries with human cells have resulted in a renewed effort to generate mouse models of IDILI based on activation of the adaptive immune system by drugs. The characteristics of recently described models (eg, amodiaquine, halothane) will be discussed. Moreover, we have developed a mouse model in which CD4+ T-cell-deficient mice with a mutation in the gene encoding MHC class II molecules resulted in liver

injury from flucloxacillin administration. CD8+ T-cells from flucloxacillin-sensitized mice proliferated, secreted IFN-γ and granzyme B and induced hepatocellular apoptosis after *ex vivo* stimulation. Details of this model of adaptive immune-mediated liver injury and its contribution to understanding IDILI mechanisms will be presented.

S 2500 Viral Preconditioning Sensitizes Livers of Mice to Injury from Exposure to Ximelagatran and TNF

P. Knolle. *Technical University of Munich, Munich, Germany.* Sponsor: R. Roth.

Viral hepatitis infections are widespread in the human population. The mechanisms by which viral infections in the liver alter the phenotype of hepatocytes and affect their response to drugs associated with IDILI, such as ximelagatran, remain unclear. Here the hypothesis that viral hepatitis increases the susceptibility to ximelagatran was tested in mice. Virally infected mouse liver is highly susceptible to ximelagatran, in particular when also exposed to TNF. This susceptibility depends on TNFR activation and results in overt liver injury and death of mice. The mechanisms for responses entail perturbations in NFkB signaling in liver cells that lead to the onset of liver apoptosis.

S 2501 Mechanisms of Cytotoxic Interaction of Diclofenac and Tumor Necrosis Factor-Alpha (TNF)

B. van de Water. *University of Leiden, Leiden, Netherlands.*

There is an increasing body of evidence that various drugs can sensitize hepatocytes to TNF-mediated killing. We now have data demonstrating that this sensitization depends on the unfolded protein response in the endoplasmic reticulum (ER) caused by drugs. Using fluorescent reporter cell lines in combination with high content imaging and RNA interference studies, the precise molecular determinants in the ER that drive this sensitization have been unraveled. In parallel to this response, drugs that sensitize towards TNF directly affect the NFkB activation. Using high throughput microscopy and RNAi approaches targeting all individual kinases and ubiquitinases, key signaling candidates have been identified that control the NFkB signaling cascade and drive the onset of cell death. These results provide a paradigm to explain mechanisms by which drug-induced cell stress responses are intricately linked to the control of pro-apoptotic cytokine signaling modules and thereby likely contribute to IDILI.

S 2502 Drugs Associated with Idiosyncratic Liver Injury Synergize with TNF and IFNg to Cause Death of Hepatocytes: Role of MAPK Activation

R. Roth, A.R. Maiuri, and P.E. Ganey. *Michigan State University, East Lansing, MI.*

Recent studies revealed that several IDILI-associated drugs from various pharmacological classes synergize with TNF to kill hepatocytes *in vitro*, and IFNg enhances the cytotoxicity. Furthermore, studies with several NSAIDs have shown roles for MAPKs in these synergistic responses. For example, NSAIDs with high IDILI liability (diclofenac, sulindac sulfide and bromfenac) synergized with TNF in causing cytotoxicity in HepG2 cells, and IFNg enhanced this interaction, whereas NSAIDs with moderate IDILI liability (ibuprofen and naproxen) synergized only with TNF. Recent explorations of mechanisms have revealed that release of calcium from intracellular stores and activation of calpain are involved in the cytotoxic response. Interestingly, inhibition of JNK or ERK reduced cytotoxicity from cytokine interactions with NSAIDs with high IDILI liability, whereas an ERK inhibitor potentiated cytotoxicity from cytokine interactions with NSAIDs with moderate IDILI liability. These findings raise the possibility that some IDILI reactions involve cytotoxic drug-cytokine synergy involving MAPKs. These novel results also suggest that, even for drugs within the same pharmacologic class, cytotoxic synergy with cytokines occurs by different mechanisms.

S 2503a Reciprocal Synergism: New Insights into Thyroid Hormone Action in Brain Development and Neurodevelopmental Toxicity

E. Fritsche[1] and P. Lein[2]. [1]*IUF - Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany and* [2]*University of California, Davis, CA.*

Clinical studies have linked inadequate thyroid hormone (TH) levels during gestation to compromised neurodevelopment that manifests as moderate to severe mental retardation, ataxia, and sensory deficits. Even mild reductions (subclinical) in maternal TH levels during early pregnancy are associated with decreased IQ in offspring. Experimental studies in rodents confirm that TH is essential for normal neurodevelopment and can influence numerous neurodevelopmental processes including neural precursor cell (NPC) and glia proliferation, neural migration, axonal and dendritic morphogenesis, synaptogenesis, and myelination. Based on this background, the concept of environmental goitrogenesis postulates that agents in the environment can produce developmental neurotoxicity if they cause a reduction in serum TH. However, recent insight into thyroid hormone action demonstrates that some tissues can autonomously regulate TH action. This recognition means that a chemical may interfere with TH action in the absence of changes in serum TH by interfering with the proper delivery of TH to target cells and/or downstream signaling mechanisms. This session will discuss ongoing research aimed at elucidating the molecular mechanisms by which TH signaling influences specific neurodevelopmental processes, understanding their conservation across *in vitro* and *in vivo* models and different species ranging from Xenopus to human, and applying this information to identify adverse outcome pathways of developmental neurotoxicity based on thyroid hormone disruption independent of changes in serum TH.

S 2503b Setting the Stage: Overview of Evidence That TH Regulates Brain Development and Postulated Mechanisms of TH Disruption in DNT

T. R. Zoeller. *University of Massachusetts, Amherst, MA.* Sponsor: E. Fritsche.

Thyroid hormone (TH) is essential for brain development; in fact all babies born in developed countries are evaluated for thyroid function at birth to prevent brain damage associated with low perinatal TH. Two assumptions have limited research in this area. First, that the thyroid system is dominated by a robust compensatory system that maintains "homeostasis". Second, that a reduction in circulating level of thyroid hormone is the sole predictor of thyroid hormone "disruption" in the context of developmental neurotoxicity (DNT). This presentation will present an overview of these concepts and describe our work that has challenged these assumptions and suggests a new avenue for research.

S 2504 Low-Level Thyroid Hormone Disruption and Neurodevelopmental Outcomes in Rodent Models

M. Gilbert. *US EPA, Research Triangle Park, NC.*

The well accepted dogma of the need for adequate supplies of thyroid hormone (TH) for optimal brain development has been largely defined from studies of severe hormone deprivation. Focus on the mechanisms and consequences of severe disruption has failed to provide necessary tools to define the potential impact of more moderate TH deficiencies on development of the brain of the fetus and neonate. Alterations in circulating levels of TH is one of the most frequently reported indices of xenobiotic interference with the thyroid system and one which can be most readily translated to exposures in humans. However, there is a distinct lack of data on structural or functional adverse outcome measures that could specifically and exclusively be tied to moderate degrees of TH disruption relevant to human physiology and within a context of fetal/neonatal exposure. This presentation will report on apical findings of altered central nervous system development and function that follow graded degrees of reduction of circulating TH in rodents using propylthiouracil, a prototype TH synthesis inhibitor upon which classic severe thyroid models systems have been based. The goal of this work is to begin to define a phenotype more likely to represent that achieved by the degree of disruption produced by xenobiotic exposure and to characterize the dose-response relationships of TH in serum and in brain and to identify the TH-dependent molecular pathways that may drive the neurodevelopmental outcome.

2505 Species-Specific Actions of TH on Fetal Neurodevelopmental Processes: An *In Vitro* Approach Using Neurospheres

E. Fritsche. *IUF – Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany.*

For elucidating TH-dependent neurodevelopmental processes in a species-specific context, time-matched neurospheres consisting of primary neural progenitor cells (NPCs) from rat (PND1), mouse (PND1) and humans (GW18) were characterized for their expression of TH signaling components as a function of days *in vitro*. The effects of T3 and T4 as well as the TH receptor antagonist NH-3 on NPC proliferation, migration, neuronal and oligodendrocyte differentiation were assessed in these *in vitro* systems and compared to neurospheres generated from TH receptor-deficient mice. Resulting TH-dependent processes were tested for TH disruption by potential endocrine active chemicals. This approach has identified TH-dependent neurodevelopmental processes in a species-specific context and established *in vitro* assays with human relevance that can be applied to at least medium throughput testing of potential thyroid hormone disrupting chemicals thereby contributing to developing AOPs that link chemical interference with thyroid hormone signaling to adverse outcomes.

2506 Species-Specific Actions of TH on Neuronal Maturation *In Vitro* and *In Vivo*

P. Lein. *University of California, Davis, CA.*

This presentation will summarize studies of developing zebrafish larvae and primary neuron-glia co-cultures from the cortices of postnatal rat and that provide novel data characterizing: (1) the ontogenetic transcriptomic profile of TH signaling components, including TH transporters, TH receptors, deiodinases, co-repressors and coregulators, and TH-dependent genes; and (2) the effects of T3, T4 and the TH receptor antagonist NH-3 on cell proliferation, apoptosis, axonal outgrowth, dendritic arborization and synaptogenesis. Data will also be presented from ongoing studies examining the effects of environmental chemicals on TH-dependent processes identified in these *in vitro* and *in vivo* models with a discussion of how these data contribute to developing AOPs that link chemical exposure to adverse neurodevelopmental outcomes independent of changes in serum TH levels.

2507 The Canary in the Coal Mine: Xenopus Tadpole Optic Tectum Development as a Biosensor for Disruption of Thyroid Hormone Signaling

C. K. Thompson. *The Scripps Research Institute, La Jolla, CA.*

Tadpoles are useful models for studying endocrine disruption because they are acutely sensitive to changes in thyroid hormone signaling. In addition, their external development allows for manipulation and observation of the early stages of brain development that are relatively inaccessible for study in mammalian systems. This presentation will review data derived from *in vivo* imaging, whole-mount immunohistochemistry, and 3D reconstruction showing that visual system development in Xenopus laevis tadpoles is dramatically affected by changes in thyroid hormone signaling. These data demonstrate that thyroid hormone acts directly on the brain to significantly affect the rate of proliferation, neuronal differentiation, dendritic arborization of visual system neurons, cell death, gene expression, and brain morphology. This model is currently being used to evaluate whether triclosan, an anti-microbial agent used in a variety of consumer household products, mimics thyroid hormone to affect brain development. Collectively, these experiments establish a baseline for the sensitivity of tadpole brain development to changes in thyroid hormone signaling, which will facilitate the development of efficient mid- to high-throughput screening to identify potential TH disruptors.

2508a The Role of Gene SLC30A10 on Manganese Homeostasis and Functional Outcomes: Implications for Homeostasis and Neurotoxicity

R. G. Lucchini[1] and D. R. Smith[2]. [1]*Icahn School of Medicine at Mount Sinai, New York, NY and* [2]*University of California, Santa Cruz, Santa Cruz, CA.*

A primary disorder of manganese homeostasis in humans was suggested by the observation of patients presenting with severe hyper-manganesemia, polycythemia, liver cirrhosis, and neurological disturbances including dystonia in absence of occupational or environmental sources of manganese intoxication. Autosomal-recessive homozygous mutations in the SLC30A10 were identified as the disease cause of this phenotype. The protein encoded by this gene has been shown to be an important manganese efflux transporter, and mutations of SLC30A10 block its intracellular trafficking and efflux activity. Early recognition of this disease is essential given that treatment with manganese chelation or oral iron supplementation, or their combination, might ameliorate symptoms and prevent progression of an otherwise potentially fatal illness. The knowledge of this new clinical disease caused by homozygous mutations of SLC30A10 gene has important implications in understanding mechanism of manganese neurotoxicity. This symposium will highlight the new evidence from human and experimental studies focusing on the influence of non-homozygous mutations in SLC30A10 on functional outcomes, parkinsonism, manganese homeostasis, and biomarkers. The impact of SLC30A10 expression will be presented on neurological performance of adolescents and elderly with environmental exposure to manganese, whereas the role of this Mn transporter will be considered as a potential determinant in a cohort of Parkinsonian cases and controls. Data on the variation of blood manganese in different human populations will be shown in relation to genetic variations of SLC30A10. The importance of new knowledge of possible mechanisms by which this manganese transporter contributes to neurotoxicity is essential to identifying early signs or pre-clinical damage in relation to manganese exposure. Cell and animal models will provide evidence of tissue- and cell-specific expression, and manganese-dependent regulation of SLC30A10. A focus on pregnancy and early-postnatal life will assess the hypothesis that altered SLC30A10 expression in maternal and infant liver function may influence manganese levels in maternal and infant bloodboth demonstrated manganese toxicity in children. The *C. elegans* model will be presented, showing how SLC30A10 overexpression protects from manganese-induced toxicity and dopaminergic neurotoxicity. Overall, this symposium will integrate innovative basic science studies with cutting-edge clinical and translational work on SLC30A10, a central player in the regulation of manganese homeostasis and the pathobiology of manganese neurotoxicity.

2508b Influence of SLC30A10 on Neurophenotypes Across the Life Span

R. G. Lucchini. *Icahn School of Medicine at Mount Sinai, New York, NY.*

Prior genetic studies showed a familial Mn-induced disease with hypermanganesemia, polycythemia, early-onset dystonia, paraparesis, or late-onset Parkinsonism, and chronic liver disease. This autosomal recessive disorder is caused by mutations of SLC30A10 (solute carrier family 30, member 10), a Mn transport protein, in absence of occupational or environmental exposure to Mn. We examined the potential impact of non-homozygous mutations of SLC30A10 on neurobehavioral outcomes measured in adolescents and elderly living in the province of Brescia, Italy, where industrial emission caused environmental exposure to manganese for more than a century. The influence of SLC30A10 mutations will be examined also in a cohort of Parkinsonian cases and controls in the same region. The influence of this gene may be persistent from early life leading to neurophenotype in the old age and potentially to neurodegeneration.

2509 SLC30A10 Determines the Mn Concentrations in Blood of Healthy Humans

K. Broberg. *Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

Manganese (Mn) is an essential nutrient in humans, but excessive exposure to Mn causes neurotoxicity. Despite homeostatic regulation, Mn concentrations in blood vary considerably among individuals. The solute carrier SLC30A10 was recently linked to a rare inherited type of hypermanganesemia in which patients accumulate Mn without any environmental exposure, indicating that it is a key regulator of Mn homeostasis. We evaluated if common single-nucleotide polymorphisms in SLC30A10 influence blood Mn concentrations and neurological pa-

rameters in human cohorts from Bangladesh, the Argentinean Andes, and Italy. We found that polymorphisms in SLC30A10 influence blood Mn concentrations in the three cohorts and that the associations may be mediated by altered levels of SLC30A10 expression. Moreover, the SNPs influenced neurological functions independent of blood Mn concentrations, suggesting that SLC30A10 could regulate brain Mn levels. These findings underscore an important role of SLC30A10 in Mn homeostasis and Mn-related disease in humans.

S 2510 Manganese-Dependent Regulation of the Efflux Transporter SLC30A10

S. Mukhopadhyay. *The University of Texas at Austin, Austin, TX.*

Homozygous mutations in SLC30A10 cause familial manganese (Mn)-induced parkinsonism. Our recent work demonstrated that the SLC30A10 protein is a cell surface localized Mn efflux transporter, and that disease-causing mutations block the ability of the transporter to traffic to the cell surface and mediate Mn efflux. We will highlight our mechanistic studies on SLC30A10 that led to its characterization as a Mn efflux transporter. Additionally, we recently identified specific residues that confer Mn transport capability to SLC30A10. These required residues reside in the N-terminal transmembrane domain as well as in the cytosolic C-terminal domain of the protein. We will discuss the role of these residues in conferring Mn transport specificity to SLC30A10. Finally, we will present new data that describes the tissue and cell-specific expression of SLC30A10 in rodent models, and provide evidence for the transcriptional regulation of SLC30A10 expression in a Mn-dependent manner.

S 2511 The Role of SLC30A10 in Body Mn Homeostasis Over Pregnancy and Early Postnatal Life: Implications for Mn Toxicity

D. R. Smith. *University of California, Santa Cruz, Santa Cruz, CA.*

Both rodent and human epidemiological studies have consistently shown that maternal blood Mn levels naturally increase over the course of pregnancy, and return to normal non-pregnant adult levels over the ensuing postpartum months. Cord blood and early postnatal infant blood Mn levels are similarly elevated, declining slowly through young adulthood. Remarkably, these peak maternal (late third trimester/parturition) and infant blood Mn levels are typically 2 – 3-fold higher than blood Mn levels associated with adult occupational exposures, likely reflecting a significantly increased physiologic need for Mn during pregnancy and postnatal development. Here, existing data documenting these natural perinatal changes are summarized, along with consideration of the potential for elevated environmental Mn exposure to augment these natural increases in body Mn. Finally, experimental rodent studies are being conducted to test the hypothesis that altered maternal and infant hepatic expression of the Mn-specific transporter SLC30A10 over pregnancy and early postnatal life is a primary mechanism contributing to these marked changes in blood Mn levels over these critical life stages. These data will have significant implications for Mn nutrition and toxicity in humans, given preliminary data presented in this symposium on the association between human SLC30A10 polymorphisms and blood Mn levels, and because maternal and infant blood Mn levels have been shown to exhibit inverted-U shaped dose-response relationships with birth weight and infant mental development index scores.

S 2512 Gene SLC30A10 and Its Role in *C. elegans*

M. Aschner. *Albert Einstein College of Medicine, New York, NY.*

We recently identified SLC30A10 to be a cell surface localized manganese (Mn) efflux transporter and demonstrated that parkinsonism causing mutations block its efflux function. In the nematode, *C. elegans*, mutations in SLC30A10 blocked the ability of the transporter to traffic Mn to the cell surface and to mediate Mn efflux. Overexpression of SLC30A10 protected worms against Mn-induced lethality and dopaminergic neurotoxicity, consistent with results in mammalian systems (HeLa cells). Furthermore, SLC30A10 expression did not protect worms against ZnSO4 toxicity, suggesting that SLC30A10 does not mediate Zn export in *C. elegans*. A blast search identified 5 potential SLC30A10 homologs in worms (cdf1, cdf2, ttm1 and toc1; sequence identity <35%); knockdown of these genes showed a tendency for increased survival after Mn exposure (although only ttm1 was statistically significant), suggesting that the worm homologs assume different function. While expression of disease-causing SLC30A10 mutations were not deleterious by themselves, worms expressing these mutants exhibited enhanced sensitivity to Mn toxicity. These studies suggest that Mn-induced neurotoxicity might be potentially mitigated by enhancement of Mn efflux via SLC30A10 (supported by NIEHS R01 ES10653).

S 2513 Using Multi- and Transgenerational Effects of Environmental Exposures in Diverse Animal Models for Assessment of Human Health Risks

K. L. Willett[1] and J. L. Freeman[2]. [1]*University of Mississippi, University, MS and* [2]*Purdue University, Lafayette, IN.*

There is a new appreciation that environmental factors acting during key developmental stages can cause irreversible changes in gene expression, and tissue structure or function, and increase the risk of developing disease. Furthermore, certain epigenetic consequences can be passed between generations, impacting offspring that were never exposed to the stressor. The way in which exposures cause multi- (into a F2 generation) and transgenerational (into a F3 generation) effects is unclear. This symposium will bring together a group of investigators who are actively engaged in determining if and by what mechanisms exposures in one generation are passed onto future subsequent generations. Speakers will highlight their work with different stressors causing multi- and/or transgenerational effects using evidence from human populations and diverse laboratory animal models with considerations for human health risk assessment. The origins of this field, as mediated by an adverse nutritional environment during prenatal and early postnatal life, resulting in transcriptional, epigenetic, and metabolic regulation and manifested in increased diabetes risk to subsequent generations in human and rodent populations, will lead off the session. The second talk will highlight the state of this research area in the field of toxicology, reviewing the various exposures reporting health effects in transgenerational studies. Current studies applying the unique aspect of the zebrafish model for multi- and transgenerational mechanistic studies will be highlighted in the next two talks relating to dioxin and atrazine, with the final talk discussing the considerations of this paradigm in a regulatory perspective for human health risk assessment and product safety. This symposium will be of interest to toxicological researchers engaged in developmental and reproductive toxicology and neurotoxicology; to those identifying epigenetic, genetic, and molecular mechanisms of adverse health outcomes in multi- and transgenerational exposure paradigms; and to those interested in health effects of exposure to endocrine disruptors, pesticides, and dioxins.

S 2514 Intergenerational Programming of Metabolic Disease: Evidence from Human Populations and Experimental Animal Models

M. E. Patti. *Harvard Medical School, Boston, MA.* Sponsor: K.L. Willett.

Common metabolic diseases, including diabetes and obesity, are the result of interactions between genes and environment. It is well-recognized that the maternal intrauterine environment is also an important modifier of this risk, with maternal type 2 diabetes, obesity, or suboptimal nutrition all promoting increased risk for metabolic syndrome in offspring. Recent data indicate that the father's metabolic and environmental history *in utero* can also impact the health of his offspring. Given the epidemic of obesity and metabolic disease in young adults reaching child-bearing age, it is critical from both a scientific and clinical perspective to identify the mechanisms responsible for these effects. In a recent study we demonstrated that first-generation male offspring mice of mothers undernourished during pregnancy (F1-UN) are not only at risk of metabolic disease themselves, but also produce second-generation (F2) offspring that develop metabolic syndrome, including increased adiposity, reduced insulin secretion, and impaired glucose tolerance as adults. Strikingly, these disease phenotypes occur without further experimental interventions during the F1 pregnancy. This indicates that a heritable non-genetic trait was created by the original *in utero* exposure and transmitted by the F1 male to his offspring, promoting a vicious cycle of disease risk. In addition, *in utero* undernutrition resulted in perturbed DNA methylation in sperm of F1-UN adult males. This is associated with altered expression of genes neighboring these differentially methylated regions in embryos of the next generation. Thus, prior paternal nutritional exposures *in utero*, as well as the current metabolic health of males, can alter epigenetic marks in sperm, contributing to disease risk in his offspring.

2515 Transgenerational Effects of Environmental Exposures in Animals: A Literature Review

V. Walker. *National Toxicology Program, Office of Health Assessment and Translation, NIEHS, Research Triangle Park, NC.* Sponsor: K.L. Willett.

Traditional dogma suggests that negative effects of early life exposures are reset such that subsequent generations are unaffected by the exposure history of parents and grandparents. However, there are reports of exposures which, in certain cases, appear to have far-reaching consequences and affect multiple generations removed from the original exposure. This phenomenon is known as "transgenerational inheritance" where there is evidence of a health effect in offspring that were not themselves exposed to a chemical or non-chemical stressor. The literature base of transgenerational effects is challenging to identify due to the wide variety of exposures considered, range of endpoints investigated, and the various ways data have been reported. The Office of Health Assessment and Translation within the National Toxicology Program recently conducted a state-of-the-science evaluation to examine the extent of the evidence for transgenerational inheritance of health effects associated with exposure to a wide range of stressors (e.g. environmental chemicals, drugs of abuse or diet) in humans or animals. The search identified over 56,000 articles, but after full text review only ~300 articles were considered relevant. The exposures and outcomes evaluated in human studies were very disparate with the majority of studies evaluating "exposures" of diet, the Holocaust and radiation. In animals, most studies evaluated exposures to chemicals, diet or pharmaceuticals and reported behavioral, developmental and reproductive outcomes. Within each of the exposure categories, there were only a few specific exposures for which there were multiple published studies for a given health effect. The evaluation assessed a subset of studies to illustrate how internal validity or risk-of-bias assessment could be applied to studies with a transgenerational study design. This presentation will discuss key conclusions of the state-of-the-science as well as the (1) challenges and strategies for identifying relevant literature; (2) risk of bias considerations of transgenerational effects or design of new studies; and (3) provide comments on data gaps, and research needs in this emerging field.

2516 Zebrafish As a Model for Adult-Onset and Transgenerational Male Infertility Due to TCDD Exposure

T. Baker. *University of Wisconsin-Madison, Madison, WI.*

We have shown that zebrafish (*Danio rerio*) are an ideal model for evaluating transgenerational effects of certain toxicants and their role in the fetal basis of adult disease. In humans, this type of question is difficult to study due to the time frame of effects. We have chosen zebrafish as a model because they are vertebrates with short generation times and consistent genetic backgrounds. Zebrafish have very modest housing needs, facilitating single and multigenerational studies with minimal time and expense. We have used this model to identify transgenerational effects of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDD) on skeletal development, sex ratio, and male-mediated decreases in reproductive capacity. Exposure to TCDD early in development produces reproductive abnormalities in adulthood and decreased reproductive capacity that persists for multiple, unexposed generations. Transgenerational inheritance of TCDD toxicity appears to be mediated through the male germline, thus gene expression in testicular tissue of the exposed adults (F0 generation) has been evaluated by microarray and qRT-PCR. We have identified changes in several testis specific genes that have also been implicated in human male infertility. The genes of interest include star, sox9b, sox9a, vtg, egr1 and sf1 (nr5a1b). Using molecular tools and the zebrafish model, our research aims to uncover critical genes and epigenetic regulation required for adverse reproductive endpoints of TCDD exposure.

2517 Multigenerational Effects of the Endocrine-Disrupting Herbicide Atrazine in Zebrafish

J. L. Freeman. *Purdue University, Lafayette, IN.*

Atrazine is a widely used agricultural herbicide in the Midwestern United States. Atrazine is regulated by the United States Environmental Protection Agency with a Maximum Contaminant Level (MCL) of 3 parts per billion (ppb) in drinking water. Atrazine is implicated as an endocrine disrupting chemical and there are questions on potential health hazards at concentrations near the MCL. For this study zebrafish were exposed to 0, 0.3, 3, or 30 ppb atrazine shortly after fertilization through the end of embryogenesis. Transcriptomic profiles immediately following the embryonic atrazine exposure identified expression alterations in genes associated with neuroendocrine function, cell cycle regulation, and carcinogenesis with morphological alterations observed in larvae from all three atrazine treatments. Using the transcriptomic data as a guide, various endpoints along the hypothalamus-pituitary-gonadal axis were evaluated in adults exposed to atrazine only during embryogenesis and in their progeny. Embryonic exposure at 3 and 30 ppb resulted in increased progesterone in adult females with increased follicular atresia in ovaries of adult females in the 30 ppb group. A decrease in spawning was also observed in the 30 ppb treatment group with their progeny having decreased head length and increased eye diameter. Transcriptomic analysis of adult female ovaries revealed alterations in genes associated with endocrine system development and function, tissue development, and behavior, while transcriptomic analysis of the adult female brain showed alterations in genes associated with nervous system development and function, tissue development, and behavior. This study provides evidence to support atrazine as an endocrine disrupting chemical causing reproductive dysfunction and molecular alterations in adult zebrafish exposed only during embryogenesis and morphological alterations in their offspring. Molecular analysis, hormone measurements, and histopathology are now being completed in the exposed adult males and in the subsequent generation.

2518 Transgenerational Effects and Implications to Product Safety Assessment

R. Rasoulpour. *Dow AgroSciences, Indianapolis, IN.*

How is the epigenome passed across generations? Could alterations within the epigenome result in persistent effects across multiple generations? How can one decouple the epigenome from the genome, transcriptome, proteome, and physiome in determining potential chemical-induced effects throughout this complexity in biology? Over ten years ago, the first studies were submitted that suggested chemical exposure of pregnant rodents can cause epigenetic changes in the (F1) conceptuses, which can be inherited by subsequent generations. To further explore this phenomenon, groups of 25 pregnant F0 CD-1 mice were administered diethylstilbestrol or estradiol from GD 9 – LD 20. Offspring were mated for two additional generations, with F1-F3 offspring evaluated for uterotrophic effects and vaginal patency, and a subset of animals evaluated at 18 months for uterine tumor incidence. The purpose of this work was to explore this phenomenon in more detail as well as explore how our, and others, transgenerational epigenetic data for areas of concordance and discordance, as well as provides thoughts on how these findings are placed within the context of product safety assessment. In particular, the current regulatory framework within product safety assessment will be discussed with a keen focus on the potential regulatory implications of these data. As causal connections between alterations in epigenetic marks and apical toxicological effects begin to be made, it raises questions on the dose response characteristics of the epigenome vs transcriptome/proteome vs classical apical end points such as clinical pathology and histopathology. As the underlying molecular changes culminate into apical end point responses, would the molecular/epigenetic point of departure be left shifted, right shifted, or lay on top of the apical end point of departure. Molecular, epigenetic, and apical end point responses have been compared across all of these species in an attempt to understand if and how epigenetics could play a role in product safety assessment and regulatory impacts.

2519a Cannabis in the Courtroom

G. Corcoran[1] and S. Bobst[2]. [1]*Wayne State University, Detroit, MI and* [2]*Nexeo Solutions LLC, The Woodlands, TX.*

Presentations in this workshop build upon 2013 and 2014 roundtable and workshop sessions that laid a broad foundation for examining the legal applications and boundaries of toxicology and the law. They highlighted the fundamental time frame dichotomy and innate tension that exists between science and the law while critically exploring the validity and limitations of toxicology evidence. The current workshop focuses specifically on the scientific and legal issues surrounding cannabis and its various forms and congeners. For suspects charged with being under the influence of cannabis, challenges for forensic laboratories include drug pharmacokinetics and pharmacodynamics as well as supporting field evidence of impairment. Epidemiology has addressed degree and time of impairment and has advanced court claims of causation, negligence, and contributory negligence in motor vehicle accidents and psychotic episodes. Many forms of synthetic cannabinoids exist, including illegal agents and those new drugs being developed as therapies. Engineered cannabinoids can greatly exceed the potency of THC and endocannabinoid yet few are scheduled as controlled substances. This poses unique difficulties for legal intervention and the protection of public health. Federal and state cannabis laws differ substantially. States with legalized marijuana must deal with toxicological uncertainty, particularly as it relates to exposure-response relationships. The expand-

ing availability of THC edibles and their consequences are considered from a poison center perspective, including dosing, delayed onset, adult deaths, and childhood near deaths. Finally, cannabis poses dilemmas for the forensic sciences as well as for toxicology and the SOT. One example is illustrated by growing efforts to establish per se blood or urine levels that would define impairment by statute, as currently exists for blood alcohol, with the potential passage of zero tolerance laws in some jurisdictions. The workshop closes with a panel discussion.

W 2519b THC from the Forensics Lab

K. Schneider. *State of Virginia Department of Forensics, Fairfax, VA.*

From the perspective of a state lab analyzing forensic specimens, especially in suspected driving under the influence of alcohol or drugs (DUI/D) cases, THC presents specific challenges both analytically as well as in offering expert opinions and testimony on the stand. The analysis of THC for legal matters begins with sample collection and preservation. Expeditiously obtaining blood samples maximizes the amount of potential THC present, which can be vital in determining impairment at the time of the alleged crime. In conjunction, storage of evidence under proper conditions (e.g., refrigeration, added preservatives, vial selection) is necessary to avoid degradation of THC and mitigate contestation of evidence integrity. Educating law enforcement personnel regarding collection and storage considerations is imperative to obtaining results that reflect the case at hand and are defensible in the courtroom. Once on the stand, the distinct pharmacokinetics and pharmacodynamics of THC complicate interpretation and assessment of impairment in human performance casework. Differences between naïve and chronic users, specifically relating to passive levels of THC in the blood and tolerance to signs of impairment, are crucial factors in THC-related forensic casework. Corroboration of field sobriety performance tests is paramount to impairment prosecution as a result of the inability to reliably correlate blood concentrations of THC to impairment. In addition, this lack of correlation has become a confounding factor in developing per se limits. When placed in combination with evolving legislation legalizing the possession and use of marijuana and THC-containing products in certain states, the role of a forensic toxicologist in THC-based casework is steadily becoming more complex.

W 2520 Epidemiological Evidence Considerations with THC in Court

M. Barrie. *Oak Ride Associated University, Oak Ridge, TN.*

Evidence from epidemiology studies is often used to support causation claims in the courtroom. Cannabis has received particular attention in several litigation areas, including motor vehicle accidents, psychosis, and degree/time of impairment. The presentation and evaluation of epidemiologic data is often limited by the study design, evidentiary rules of admission and causation, and is possibly misrepresented and confusing in the courtroom. Courts have applied various approaches for determining the relevance and validity of epidemiological evidence in court, particularly with respect to causation, negligence, and contributory negligence. Specific reviews of some of the evaluation factors will be discussed, with particular attention to some cases involving motor vehicle accidents and psychosis associated with consumption of cannabis / marijuana. For example, case reports have been cited for propositions of causal inference where the application of the Hill Criteria for determinants of association may not be satisfied. From the standpoint of expert opinion challenges and admissibility, the use of case reports and case series will be discussed from the epidemiologic perspective on causation and association. Case analysis will identify some areas were the use of an epidemiologic investigations and data to support causation maybe a strong or weak argument, depending on the circumstances of the case. The use of epidemiologic studies and data need to be understood in terms of their ability to address issues of causation (versus association), study design limitations (cross-sectional investigations versus case-control and cohort investigations) and statistical significant (confidence intervals).

W 2521 THC Cases in State versus Federal, Different Case Issues

L. Plunkett. *Integrative Biostrategies, Houston, TX.*

Legalization of marijuana in some states mean different rules may apply to the evaluation of marijuana evidence for a testifying expert toxicologist. There are unique challenges presented by the legalization that are related to the pharmacokinetics and pharmacodynamics of the complex mixture of active ingredients. Included among these are the need to delineate sound exposure-response relationships for impairment, the presence of various psychoactive components with differing levels and types of activity, and the challenges posed by the long retention time of marijuana metabolites in body tissues. Relevant state regulations along with forensic evidence examples will be used to illustrate the data gaps that exist in terms of understanding how to interpret blood and urine data and correlate these levels with human behavior. Examples will relate to two of the first states to legalize marijuana, Washington and Colorado, and the lack of adequate standards for identifying impaired drivers, as well as the problem of the large variety of psychoactive substances other than tetrahydrocannabinol. The discussion will be focused on the need to develop consistent standards for impairment due to marijuana ingestion and inhalation and the need to recognize that substances other than tetrahydrocannabinol are at issue.

W 2522 General Population Experiences with THC Edibles–A New Kind of High

C. Hoyte. *Rocky Mountain Poison Control Center, Denver, CO.*
Sponsor: S. Bobst.

With the legalization of recreational marijuana in Colorado, there are many THC infused edibles that are available for consumption by the general public. These edibles range from soft drinks, to candies, to baked goods – with new infused edibles being introduced almost daily. Most people have very limited experience with these items, the suggested dosing amounts can vary from item to item and the delayed onset of effects can lead to over consumption and overdosing. Calls to the regional poison control center have increased significantly since legalization in Colorado and the type of clinical effects reported are different from those seen with smoking marijuana. Edibles have been implicated in adult deaths and near deaths in small children. Unintentional exposures to children are increasing, and can lead to legal issues such as child endangerment and other legal actions. Collectively, these events pose unique challenges for responders, and likely will become more common as more states consider the relaxation of cannabis laws.

W 2523 The Legal Dragnet for Elusive "THC"

R. T. Kennedy. *New Mexico Court of Appeals, Albuquerque, NM.*
Sponsor: S. Bobst.

THC, along with its metabolites and illegitimate synthesized analogs and doppelgangers continue to frustrate and confuse the legal system in both criminal and civil cases. Driving Under the Influence (DUI) drug and designer drug legislation provide two salient examples. In the former, relating ingestion to a relevant effect on driving impairment compelled policy makers to turn away from per se limits. The result was consensus that zero-tolerance laws criminalizing mere presence of THC or any of its metabolites (even non-psychoactive) in blood or urine were the only logical solution given the pharmacology and toxicology of THC, and its illegality. In states where medical marijuana is permitted, zero-tolerance owing to illegality is anachronistic, and arbitrary limits are again considered. On the other side of criminal law, "synthetic THC" and its analogues are changing their chemical structures as quickly as legislatures can criminalize them. A recent collection of mass spectra of "designer drugs" advertises "109 cannabinoids" among "21,013 mass spectra, over 700 more spectra than the 2014 edition" (1) The effect is two-fold: first, the law is not adept at dealing with marginally modified molecules, either in concept or definition. Second, those molecules in many instances are hitting the streets with deadly effect, and the slow-moving legal effort to deal with them is overwhelming both law enforcement and the courts. Toxicological and pharmacological data on effects and dangers will lag, as will establishing legal responses to, and liability for, the dangers posed by these drugs. Reference 1) Mass-mailed advance advertising solicitation: P Rosner, Mass Spectra of Designer Drugs 2015, Wiley-VCH Publishers, 2015.

W 2524 Read-Across: Building Scientific Confidence in the Development and Evaluation of Read-Across for Regulatory Purposes Using Tox21 Approaches

R. A. Becker[1] and G. Patlewicz[2]. [1]*American Chemical Council, Washington, DC and* [2]*US EPA, Research Triangle Park, NC.*

Read-across, whereby chemical hazard data from data-rich chemicals are used to predict the hazards of another chemical lacking such data, is a well-established approach in chemical management. Even as read-across continues to generate considerable interest as a practical data gap-filling technique in category and analogue approaches for regu-

latory purposes, its acceptance by regulatory agencies remains a challenge. In an effort to enhance regulatory acceptance of read-across, several organizations have begun to develop systematic frameworks to characterize read-across justifications and make explicit the uncertainties and assumptions relied upon. Such efforts are intended to build scientific confidence associated with read-across for regulatory purposes. A growing strategy for reducing uncertainty in read-across is to incorporate Tox21 approaches in substantiating category rationales or their associated read-across justifications. For example, mechanistic information captured within Adverse Outcome Pathways (AOPs) could be used in category development as part of Integrated Approaches to Testing and Assessment (IATA). Additionally, data generated under the ToxCast program can be used in supporting read-across justifications for new and existing regulatory categories. This workshop will foster a cross-stakeholder discussion on what the key issues thwarting regulatory acceptance are and what progress has been made in investigating the utility of mechanistic information to meet different regulatory decision-making needs. The workshop will comprise a series of highly focused presentations that evaluate the development of chemical categories and associated read-across and their interpretation in regulatory decision-making in chemical risk assessment. Each speaker presenting a case study will be asked to consider how they are evaluating the confidence level of the read-across in terms of the supporting mechanistic data and whether, if appropriate, this anchors to a specific pathway(s) (e.g., AOPs). The session will be of broad interest to investigators and regulators across environmental, industrial, consumer products, and pharmaceutical toxicology that perform human and environmental risk assessment.

W 2525 Making the Key Issues Driving Uncertainty in Read-Across Explicit Using the RAAF–Opportunities for Enhancement with Modern Approaches

D. Knight. *European Chemicals Agency, Helsinki, Finland.* Sponsor: R. Becker.

The European Chemicals Agency (ECHA) administers the EU chemicals legislation covering general chemicals. In the evaluation of registration dossiers submitted by registrants, ECHA has to examine the scientific justifications for read-across and chemical categories: the Read-across Assessment Framework (RAAF) presents a structured tool for ECHA evaluators. The RAAF encourages the use of mechanistic information to substantiate the read-across justification: this reminds registrants of the possibility to include evidence from the application of modern approaches in toxicology, such as adverse outcome pathways (AOPs) and omic technologies. The possibility to strengthen read-across justification in this way was investigated in an EU 7th Framework Research Initiative named 'Safety Evaluation Ultimately Replacing Animal Testing' (SEURAT-1). The SEURAT-1 read-across case studies demonstrate that the robustness of read-across of repeated-dose oral toxicity can be improved using SEURAT-1 and ToxCast evidence: Scenario I: a category of direct toxicants (i.e. non-metabolising), perfluorinated fatty acids (PFAAs); Scenario II: a category of indirect toxicants (i.e. requiring metabolic activation), ß-unsaturated alcohols (allylic alcohols); Scenario III: a category of low toxicity substances, primary saturated alcohols and Scenario IV: a category that 'breaks' into different toxicity subgroups, alkyl phenols.

W 2526 Actualizing the OECD Revised Guidance on Grouping of Chemicals with Practical Examples

J. deKnecht. *OECD, Paris, France.* Sponsor: R. Becker.

New ways of grouping chemicals into toxicologically meaningful categories include grouping on the basis of an adverse outcome pathway (AOP), which itself is a framework for describing the key events at different levels of biological organisation and their causal relationship to the *in vivo* regulatory endpoint under consideration. Since the publication of the revised grouping guidance (OECD, 2014) and the workshop on the development and application of Integrated Approaches to Testing and Assessment (IATA), the OECD work programme under the auspices of Task Force for Hazard Assessment (TFHA) has called for case studies to actualise the practical utility of incorporating mechanistic information by way of AOPs into category development to support endpoint specific read-across. This presentation will describe the insights gained so far (e.g. general principles of essential information for regulatory application of IATA and the type of data that can be used to strengthen the confidence in reading across) and comment on any specific challenges (e.g. uncertainty communication) that would lend themselves to further research and study.

W 2527 Propylene Glycol Ethers: A Read-Across Example for Low-Toxicity Chemicals

N. Ball. *The Dow Chemical Company, Midland, MI.* Sponsor: R. Becker.

A significant challenge for acceptance of read-across is for chemicals with general low or no toxicity even though a large number of chemicals fall under this designation. Therefore, the P-series (propylene glycol-series) glycol ethers have been proposed as ideal candidates for a read-across case study. They have a low order of toxicity, limited primarily to organ weight changes in the liver and kidney. The category justification for the grouping of P-series glycol ethers and subsequent use of read-across is supported by a large database of *in vivo* studies, however there are still opportunities to generate additional information to support read-across with a more mechanistic based justification, or a broader 'biological-profiling' approach. Specifically, using *in vitro* cell systems (hepatocytes, cardiomyocytes and lymphocytes) biological activity data have been generated using the P-series glycol ethers in order to demonstrate that the category members not only have similar structural characteristics, but also similar biological activity. In addition, data on a structurally related, but toxicologically distinct category of substances (Ethylene glycol-series glycol ethers) have been generated using the same methodology to demonstrate the ability of the approach to distinguish between toxicologically diverse substances as well as group toxicologically similar substances. The presentation will demonstrate how new approaches can be used to add to the weight of evidence substantiating the validity for read-across in the P-series glycol ethers.

W 2528 Do Tox21 Approaches Such as Data from ToxCast Enhance Existing OECD Chemical Categories?

G. Patlewicz. *US EPA, Research Triangle Park, NC.* Sponsor: R. Becker.

Acceptance of read-across is an ongoing challenge and several efforts are underway to identify and address major uncertainties associated with read-across. Several approaches have been proposed but to date few case studies if any have evaluated how Tox21 approaches may be instructive in substantiating category rationales, or indeed their associated read-across justifications. Here we have identified a handful of former OECD HPV categories and evaluated to what extent data from HTS assays notably that generated through ToxCast are helpful in strengthening the rationale underlying those categories and their associated read-across. A handful of categories were identified based on the overlap between the HPV chemicals and the ToxCast inventory. Those selected included the ethylene glycols and primary aliphatic alcohols categories. The findings so far are mixed, highlighting the importance of carefully interpreting the ToxCast data in its appropriate context.
This abstract does not necessarily reflect US EPA policy.

W 2529 CAAT and the Work of the Read-Across Steering Group

T. Hartung. *John Hopkins CAAT, Baltimore, MD.*

The CAAT read-across steering group was established in 2014 with an ambitious agenda to drive efforts in the acceptance of read-across for regulatory purposes, notably those envisaged for the REACH 2018 deadline. A number of areas were identified to work on including describing the state of the art of read-across ("Good Read-Across Practice"). This includes advice on how to perform read-across for complex mixtures such as petrochemicals (substances of unknown or variable composition, complex reaction products or biological materials collectively called UVCBs) particularly in a regulatory environment focused on structural similarity. A key aspect for UVCB and defined substances is, how to apply biological activity data to support read-across, which can originate from high-throughput testing, omics profiling or targeted *in vitro* testing of a group of substances ("test-across"). A central topic was assessing and expressing uncertainties in read-across using measures of chemical and biological similarity of the chemical neighbours with available test data. Lastly, a sharing scheme for examples submitted under REACH and responses received from ECHA was established. This presentation provides a status on the learnings and deliverables under the umbrella of this steering group. It also summarises the feedback received on the jointly developed Good Read-Across Practice in stakeholder fora in Washington and Brussels in early 2016.

2530 Safety Assessment of Topically Exposed Cosmetic Ingredients: Lessons Learned

A. Schepky[1] and B. Blaauboer[2]. [1]*Beiersdorf AG, Hamburg, Germany and* [2]*Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands.*

The Cosmetics Europe Program aims to enable *in vitro*-only safety assessments of cosmetic ingredients performed on the basis of both hazard characterization and exposure assessment. The focus is on the development of models linking external exposure dose to local skin and internal exposure, and potential target organ toxicity after topical application. As a result of the 7th Amendment to the Cosmetic Directive, the use of animals for such studies is banned in the EU, and therefore information regarding systemic exposure of dermally applied chemicals must be derived from *in vitro* and/or *in silico* methods. This session provides an overview of three Task Force Projects, and how results will help in the animal-free safety assessment for skin sensitization and genotoxicity of topically applied chemicals. The session is rounded off with a regulators perspective on how we may gain acceptance of predictive approaches to the safety assessment of cosmetic ingredients by regulators themselves, as well as end-users and the consumer. The Skin Bioavailability and Metabolism (Skin BM) Task Force was set up to improve the measurement and prediction of the bioavailability of topically exposed compounds, with a specific focus on endpoints such as skin sensitization and genotoxicity. The Skin BM project has generated data from standardized dermal-based assays for a set of physicochemically diverse chemicals, which are relevant to cosmetics and dermal toxicities. An example of how these data can improve the predictive capacity of an *in silico* dermal penetration model will be presented. In addition, we will demonstrate how bioavailability information (including dermal absorption and metabolism) can be utilized when assessing outcomes from skin sensitization assays and dermal genotoxicity assays (using 3D skin). The Skin Tolerance Task Force has studied the complex biology of skin sensitization and conducted a screen and evaluation of different *in vitro* test methods, which provide insight into sensitizing characteristics of chemicals. Case studies demonstrating how the safety of topically applied cosmetic ingredients, e.g., resorcinol, cinnamic acid, or hair dyes, can be supported with this concept will be introduced. An evaluation of multiple integrated approaches to testing and assessment of skin sensitization will be presented, and a potential path toward regulatory acceptance of an optimized strategy will be discussed. The Genotoxicity Task Force has led three projects to help improve the predictive capacity of current *in vitro* genotoxicity assays and develop new *in vitro* models as follow-up alternatives to positive outcomes in the initial test battery. Work from these Task Forces will ultimately lead to a testing strategy to enable cosmetic industries to conduct safety assessments of chemicals without the use of animals.

2531 Generation of Dermal Bioavailability Data Using *In Vitro* Studies for Local Dermal Concentrations and *In Silico* Models

A. Schepky. *Beiersdorf AG, Hamburg, Germany.*

The Cosmetics Europe Skin BM Task Force is using *in vitro* models to determine of the fate of 50 compounds (e.g. sensitizers, genotoxins) after application to the skin. The 8 assays (including skin penetration and metabolism, partition/diffusion coefficients in different skin layers and peptide binding) have been harmonized (dose, solvent etc) so that the data can be compared across chemicals. Results provide relevant and standardized information on the local dermal and systemic concentrations (e.g. input into PBPK models for Cmax and AUC which can then be used in combination with cheminformatics and AOPs to evaluate systemic toxicity), of chemicals applied to the skin.

2532 Skin Penetration *In Silico* Modeling–Use of *In Vitro* Data to Improve Kasting Model

G. Kasting. *University of Cincinnati, Cincinnati, OH.* Sponsor: A. Schepky.

Skin absorption can be measured using *in vitro* methods; however, they are expensive and time-consuming; therefore, predictions of skin absorption using *in silico* models would help to address this. Parameters that are measured experimentally are currently lacking and are needed to improve the predictivity of an *in silico* model. The data generated by the Skin BM Task Force have been used to try to develop further an *in silico* model based on the lipid pathway.

2533 Skin Sensitization Testing Strategy Evaluation

N. C. Kleinstreuer. *Integrated Laboratory Systems, Inc. Contractor supporting the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), Research Triangle Park, NC.*

The Skin Tolerance Task Force has generated data on >100 chemicals in multiple *in vitro* skin sensitization assays that have been screened and assessed. These assays, together with certain *in silico* predictions, are key components of various integrated approaches to testing and assessment (IATA). Murine LLNA and human sensitization data were used to evaluate the performance of testing strategies in both hazard and potency characterization. IATAs examined include Bayesian models, artificial neural networks, support vector machine models, and decision trees. An optimized strategy will be presented that incorporates current mechanistic understanding outlined in the OECD Adverse Outcome Pathway for Skin Sensitization.

2534 Method Evaluation Process and Outcome for Skin Sensitization

N. Alépée. *L'Oréal Research & Innovation, Aulnay Sous Bois, France.* Sponsor: A. Schepky.

The Skin Tolerance Task Force compiled case Studies to demonstrate how various Integrated Approaches to Testing and Assessment (IATA), in combination with e.g. exposure considerations and *in silico* approaches, can be engaged to support meaningful risk assessments. The importance of (1) investigating the link between the penetration-dependent free local concentration of chemicals and their skin sensitization potential is discussed and (2) chemicals that can cause skin sensitization either directly or as an outcome of bio-activation (e.g. via skin metabolism) to a reactive chemical will be discussed. Available information on metabolism and on skin penetration may trigger modifications of IATA's and lead to further fine-tuning. These case studies will show how knowledge of the bioavailability, including the extent of skin metabolism, can help to interpret skin sensitization potential.

2535 Use of Bioavailability Data to Interpret 3D Skin Genotoxicity Assay Outcomes

S. Pfuhler. *The Procter & Gamble Co., Cincinnati, OH.*

Cosmetics Europe has funded projects to develop and evaluate dermal *in vitro* models for genotoxicity by combining the use of 3D reconstructed skin (RS) tissues with micronucleus and Comet assays readouts. These provide a more realistic model for dermally applied chemicals, such as cosmetics, and can be used as a follow-up for positive results from the standard *in vitro* genotoxicity battery. Both protocols include a longer exposure time, especially for chemicals that required metabolism to the ultimate genotoxin. Case studies will be presented demonstrating that information on dermal penetration and, even more importantly, metabolism of promutagens facilitates the interpretation of the outcome of the 3D skin genotoxicity assays.

2536 Gaining Acceptance of Predictive Approaches to the Safety Assessment of Cosmetic Ingredients

M. P. Whelan. *EURL/ECVAM European Commission Joint Research Centre, Ispra, Italy.* Sponsor: A. Schepky.

The European Union first implemented a ban on animal testing of finished cosmetic products over a decade ago. Further steps to phase out animal testing followed, which culminated in the establishment, under the Cosmetics Regulation, of a marketing ban on cosmetic products and ingredients which have been tested on animals after the 13th March 2013. Although in certain cases toxicological data on cosmetic ingredients can be obtained from animal tests conducted in order to satisfy information requirements under other pieces of EU chemicals legislation (e.g. REACH), the cosmetics sector is essentially faced with the challenge of carrying out a safety assessment without resorting to an animal test where data gaps exist. However, toxicology science has advanced tremendously and offers a new generation of *in vitro* tools and computer models that can be used to predict toxicity rather than just observe it. Importantly too, predictive approaches reveal and exploit mechanistic understanding of where a chemical ends up in an organism and why it might be toxic under certain conditions, opening the door to more

tailored, ingredient-specific approaches to safety assessment. But as the science advances and the solutions emerge, we need to ensure that alternative (to animal testing) approaches are suitably packaged, evaluated, documented and demonstrated to pave the way for widespread acceptance by regulators, end-users and the consumer.

PL 2537 Histopathological Findings After 12 Months Inhalation to Nano Ceria

L. Ma-Hock[3], H. Ernst[2], S. Gröters[3], S. Rittinghaus[2], J. Keller[3], B. van Ravenzwaay[3], D. Schaudien[2] and R. Landsiedel[3]. [1]*Department of Pathology, Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany;* [2]*Department of Toxicology, Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany and* [3]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

Lung carcinogenicity and putative systemic effects of low-dose lifetime inhalation exposure to biopersistent nanoparticles were examined in a chronic/carcinogenicity inhalation study performed according to OECD test guideline no. 453 with several protocol extensions. Female rats (100/group) were exposed to cerium dioxide (NM-212, 0.1; 0.3; 1; 3 mg/m^3) for two years; a control group was exposed to clean air. After one year exposure, 42 µg cerium dioxide/lung was found in animals exposed to 0.1 mg/m^3 and 2.6 mg/lung in animals exposed to 3 mg/m^3. Histological examination of lungs revealed several adverse and non-adverse effects in the lung. The non-adverse effects comprised accumulation of particle-laden macrophages in alveolar/interstitial areas and in the BALT, particle-laden syncytial giant cells in the BALT and bronchiolo-alveolar hyperplasia (alveolar bronchiolization). The adverse effects included (mixed) alveolar/interstitial inflammatory cell infiltration, alveolar/interstitial granulomatous inflammation, interstitial fibrosis and alveolar lipoproteinosis. The incidence and severity of the effects were concentration-related. Alveolar lipoproteinosis was not observed at lower concentrations of 0.1 and 0.3 mg/m^3 CeO2. Neither pre-neoplastic nor neoplastic changes were observed after 12-months exposure. A no observed adverse effect concentration could not be established in this study. The comprehensive histopathological examinations of lungs and other tissues, after 24 months of exposure will be finalized in 2017.
References: Keller, Jana, et al. "Time course of lung retention and toxicity of inhaled particles: short-term exposure to nano-Ceria." Archives of toxicology 88.11 (2014): 2033-2059.

PL 2538 Coo and La2O3 Nanoparticle-Induced Pulmonary Response in Mice After Whole-Body Inhalation Exposure

J. D. Sisler[1], W. McKinney[1], A. Mihalchik[1], R. Mercer[1], J. Shaffer[1], M. E. Andrew[1], M. Wolfarth[1], D. Porter[1], L. Battelli[1], B. T. Chen[1], V. Castranova[2] and Y. Qian[1]. [1]*National Institute of Occupational Safety and Health, Morgantown, WV and* [2]*Department of Pharmaceutical Sciences, WVU, Morgantown, WV.*

Metal oxide nanoparticles have the unique property of semi-conduction and can serve as conduits for electron transfer between aqueous reactants. These semi-conducting properties are implicated in adverse health effects. Two metal oxide nanoparticles with different semi-conductor properties, cobalt monoxoide (CoO) and lanthanum oxide (La2O3) may pose different toxicological potentials *in vivo* and *in vitro*. Our previous *in vitro* study showed that CoO nanoparticles induce a more potent toxicological response in human small airway epithelial cells than La2O3 nanoparticles. The current study determined CoO and La2O3 nanoparticles-induced pulmonary response in mice after whole-body inhalation exposure. Mice were exposed to 10 or 30 mg/m3, for 6 h per day over 4 days and were examined at 1, 7 and 56 days post-exposure. Both CoO and La2O3 nanoparticles were present in the lung at 1, 7 and 56 days post exposure. CoO caused greater lactate dehydrogenase in the bronchoalveolar fluid compared to La2O3 at 1 day post exposure at both doses. La2O3 had elevated numbers of macrophages, lymphocytes, neutrophils and eosinophils in the bronchoalveolar fluid compared to CoO nanoparticles at both doses and all post-exposure time points. Histopathological results show that there was acute pulmonary inflammation at 1 day post-exposure for CoO and an inflammation at 7 day post exposure for La2O3 Fibrosis was not observed at 56 day post-exposure at 10 and 30 mg/m3. Mice exposed to CoO and La2O3 have different expression patterns of pro-inflammatory cytokines at all post exposure time points and doses. Taken together, the results demonstrate that CoO nanoparticles induce more overall acute pulmonary toxicity where as La2O3 nanoparticles caused chronic inflammation. Moreover, this study may fill the gap between *in vivo* and *in vitro* nanoparticle-induced toxicity studies and risk assessment.

PL 2539 Repeated Inhalation Exposure to Amorphous Silica Nanoparticle-Containing Slurries: Derivation of Workplace Exposure Limits for the Electronics Industry

A. Elder[2], A. Kennell[2], R. Gelein[2], S. Brenner[1] and G. Oberdörster[2]. [1]*Colleges of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY and* [2]*Environmental Medicine, University of Rochester, Rochester, NY.*

Background: Chemical mechanical planarization using amorphous silica nanoparticle (NP)-containing slurries is done in the semiconductor industry, creating the potential for the aerosolization of the slurry constituents and subsequent exposures. Based on previous acute rat inhalation exposure studies, we hypothesized that repeated inhalation exposure to silica NP (31 nm) slurry would result in dose-dependent and persistent pulmonary inflammatory reactions in rats. Methods: The slurry was diluted in water, ultrasonically nebulized, and male F-344 rats (200-300 g) were exposed via whole-body inhalation to the dry aerosol for 4 hrs/day, 5 days/week for 4 weeks (total of 19 exposure days) at airborne SiO2 concentrations of 0.2, 1.0, or 4.7 mg/m3 (MMAD, 0.38-0.5 µm; GSD, 1.8-2.4). Controls were exposed to filtered air. Results: Retained lung burdens at 1 day post-exposure (PE) were 14±3, 47±9, and 196±7 µg of SiO2 for the low, mid, and high doses, respectively. These values are lower than what is predicted, suggesting high biosolubility of the SiO2 particles. Lung tissue was harvested from separate groups of rats at 1, 7, and 27 days PE to evaluate inflammatory responses. At 1 day PE, the mid and high concentrations produced statistically significant elevations in lavage neutrophils (5.8%±1.7; 33.9%±1.8) and other inflammatory parameters; although the magnitude of the responses decreased during recovery, they were still higher than controls at 27 days PE. In contrast, the lowest concentration did not produce any significant changes in inflammatory parameters and is, thus, a no-observed-adverse-effect concentration for this study. Conclusions: Using dosimetric extrapolation modeling based on alveolar surface areas and lung weights, human-equivalent exposure concentrations that should be protective for workplace exposures ranging from 0.2 mg/m3 (lung weight basis) to 0.8 mg/m3 (alveolar surface area basis), measured as SiO2, were determined. This research was funded by NIH P30 ES01247 and SUNY Research Foundation subaward 13-15.

PL 2540 Investigating *In Vivo* Bioprocessing of Engineered Nanoparticles (NP): A Novel Approach in Nanotoxicology

U. M. Graham[1], G. Oberdorster[2] and A. Elder[2]. [1]*Center for Applied Energy Research, University of Kentucky, Lexington, KY and* [2]*Environmental Medicine, University of Rochester, Rochester, NY.*

Background: By using advanced imaging technologies (1 nm spatial resolution) and simultaneous elemental analysis with acquisition of material phase changes and analysis of oxidation states of retained NP in tissue, we define "*in vivo* processing" as the dynamic chemical and/or physical breakdown of the NP at the cellular and subcellular level. The goal of our studies is to investigate cellular interactions of NP in target tissues associated with their retention and biological/toxicological effects. Methods: Groups of rats were exposed to amorphous SiO2 NP aerosols for 4 hrs/day, 5 days/week for 4 weeks with a 27 day post-exposure observation period at three concentrations. Dose-dependent pulmonary inflammation was found in the rats. Prior analysis of the clearance kinetics using modeling of retained lung burden showed significant *in vivo* solubility which raises the question about underlying cellular mechanisms that result in the instability of the particles and toxicity. Lung tissue was fixed and thin sections were prepared for high resolution imaging. Results: Examination of phagocytosed SiO2 NP in phagolysosomes of alveolar macrophages showed significant *in vivo* breakdown and transformation of the NP after inhalation. A comparison with the pristine precursor SiO2 NP showed that the original spherical morphology of ~20-40 nm size was not retained in the lung. Instead, the NP exhibited dissolution patterns and secondary reaction zones which formed around partially dissolving SiO2 NP. We discovered that the *in vivo* processing led to second generation NP of ultra-small sizes (<4 nm) which resulted in a much greater surface area. Elemental mapping of the *in vivo* processed SiO2 NP showed an association of sulfur and phosphor-rich zones in the immediate vicinity of the bioprocessed NP. Moreover, ultra-high resolution analysis of the reaction zones around most SiO2 NP in phagolysosomes show breakdown patterns, void spaces and significant pore formation, suggesting that there are continuing processes that release and relocate the SiO2 NP which results in the observed reaction zones. Conclusion: Further detailing the physico-chemical changes during bioprocessing of NP may be an effective tool in understanding their subcellular and temporal fate that controls toxicity.

2541 Extra-pulmonary Translocation of CeO2 Nanoparticles: From Lung to Liver After Subacute and Chronic Low Dose Inhalation

J. Brunner[2], J. Tentschert[2], H. Jungnickel[2], P. Laux[2], L. Ma-Hock[3], J. Keller[3], T. Gebel[1], A. Luch[2] and R. Landsiedel[3]. [1]*German Federal Institute for Occupational Safety and Health, Dortmund, Germany;* [2]*Department of Chemical & Product Safety, German Federal Institute for Risk Assessment, Berlin, Germany and* [3]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany.* Sponsor: A. Haase.

Cerium dioxide (CeO2), a representative of granular, biopersistent particles without known specific toxicity (GBP), was examined on lung carcinogenicity and putative systemic effects of low-dose life-time inhalation exposure in a chronic inhalation study according to OECD TG 453. So far, no definite conclusions on the biokinetics of GBP nanoparticles under the conditions of chronic inhalation could be drawn, despite the numerous *in vitro* and *in vivo* studies. Furthermore, concerns about adverse impacts on extra-pulmonary organs, especially metabolizing organs like liver, are emerging for CeO2 due to its alternating redox character. For that reason we determined the burden of the lungs and extra-pulmonary organs according to their contents of CeO2 nanoparticles (NM-212) resulting either from subacute inhalation (study according to OECD TG 412) or from chronic low-dose inhalation (study according to OECD TG 453). The aim of these studies was to examine the clearance, translocation and excretion of CeO2. The maximum CeO2 lung burden was determined with 5.88 mg after 24 months of exposure to an aerosol concentration of 3 mg/m^3. Additionally, tracheobronchial and mediastinal lymph nodes contained high levels of CeO2, possibly because these lymph nodes drain the lung. Since in liver a maximum content of 0.0167 mg CeO2 was detected, we have investigated the distribution pattern of the substance with Time-of-Flight Secondary Ion Mass Spectrometry. This work is part of the NANoREG project, jointly funded by the European Union, German Federal Institute for Risk Assessment (BfR), BASF SE, German Federal Institute for Occupational Safety and Health (BAuA) and German Federal Environmental Agency (UBA).

2542 Investigation of CeO2 Nanoparticle Toxicity in a 90-Day Inhalation Test

D. Schwotzer, M. Niehof, T. Hansen, S. Schuchardt, T. Tillmann, H. Ernst and O. Creutzenberg. *Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany.* Sponsor: C. Dasenbrock.

Cerium dioxide is a frequently used nanoscaled dust. As additive in diesel fuel it is released into the environment and inhaled by organisms. While little is known about the adverse effects of CeO2 nanoparticle inhalation, this project aimed on estimating the effects of nanoscaled CeO2 following a 90-day nose-only inhalation in the rat. To determine early indicators of genotoxic and carcinogenic effects the results of this study are compared to a combined chronic inhalation toxicity and carcinogenicity study with the same test items (BASF, Ludwigshafen, Germany). Focusing on repeated inhalation of low nanoparticle levels, rats were exposed to CeO2 in concentrations of 0, 0.1, 0.3, 1 and 3 mg/cm^3 (6 h/day, 5 days/week, 13 weeks). Exposure to a high concentration of nanoscaled barium sulfate (50 mg/m^3) was additionally investigated. The 90-day exposure was conducted under the same conditions used in the chronic BASF test. Animals were sacrificed after one and 28 days of exposure, as well as after one, 28 and 90 days post-exposure. Besides obligatory endpoints (OECD guideline 413), type II pneumocytes were isolated for gene expression analyses. Pathway arrays for genotoxicity, oxidative stress, inflammation, apoptosis and lung cancer were used to identify marker genes, whose mRNA expression is markedly affected by nanoparticle exposure. In addition, retention of the test items in lungs was determined. - Analyses of bronchoalveolar lavage fluids (BALF) indicate a time and dose-dependent increase of inflammatory cells in the lung up to the end of exposure. Respective values decrease during post-exposure. - The present results suggest an impact of CeO2 nanoparticles on cellular systems especially in the lung. The relevance of those effects and its relation to the development of tumors will be assessed in the context of the various endpoints applied. This project is funded by the German Federal Ministry of Education and Research (BMBF) - 03X0149A.

2543 Acute and Subchronic Oral Toxicity Studies in Rats with Different Nanoscale and Pigment Grade Titanium Dioxide Test Particles

D. B. Warheit[1], E. M. Donner[2] and S. C. Brown[1]. [1]*Chemours Company, Wilmington, DE and* [2]*DuPont Company, Newark, DE.*

Data generated using standardized testing protocols for toxicity studies generally provide reproducible and reliable results for establishing safe levels. The findings of three OECD guideline-type oral toxicity studies of different duration in rats are summarized. Each study evaluated different titanium dioxide (TiO2) particles of varying sizes and surface coatings. Moreover, each study result demonstrated an absence of any TiO2 -related hazards. To summarize the findings: 1) In a subchronic 90-day study (OECD TG 408), groups of young adult male and female rats were dosed with rutile-type, surface-coated pigment-grade TiO2 test particles (d50 = 145 nm - 21% nanoparticles by particle number criteria) by oral gavage. The NO Adverse Effect Level (NOAEL) for both male and female rats in this study was 1000 mg/kg bw/day, the highest dose tested; based on a lack of TiO2 particle-related adverse effects on any in-life, clinical pathology, or anatomic/microscopic pathology parameters; 2) In a 28-day repeated-dose oral toxicity study (OECD TG 407), groups of young adult male rats were administered daily doses of two rutile-type, uncoated, pigment-grade TiO2 test particles (d50 = 173 nm by number) by oral gavage at doses of 24,000 mg/kg bw/day. There were no adverse effects measured during or at the end of the exposure period; and the NOAEL was 24,000 mg/kg bw/day; 3) In an acute oral toxicity study (OECD TG 425), female rats were administered a single oral exposure of surface-treated rutile/anatase nanoscale TiO2 particles (d50 = 73 nm by number) with doses up to 5000 mg/kg and investigated over a 14-day post-exposure period. Under the conditions of this study, the oral LD50 for the test substance was >5000 mg/kg bw. In summary, the results from these three toxicity studies - each with different TiO2 particulate-types, demonstrated an absence of adverse toxicological effects. In addition, an attempt is made to reconcile the complex issue of particle size distribution as it relates to measurements of nanoscale and pigment-grade TiO2 particles. It is clear that the particle-size distribution for pigment-grade TiO2, contains a small ("tail") component of nanoscale particles (i.e., 21% by particle number and <1% by weight in the test material used in the 90-day study). Based upon the results of 3 studies, there appears to be no adverse oral toxicity impact contributed by the nanoscale component of the TiO2 test material.

2544 A Decision-Making Framework for the Grouping and Testing of Nanomaterials (DF4nano Grouping)

R. Landsiedel[8], J. H. Arts[13], M. Hadi[11], M.-A. Irfan[8], A. Keene[1], R. Kreiling[2], D. Lyon[12], M. Maier[3], K. Michel[4], N. Neubauer[8], T. Petry[7], U. G. Sauer[5], K. Wiench[8], W. Wohlleben[8] and D. Warheit[6]. [1]*Afton Chemical, Richmond, VA;* [2]*Clariant Produkte GmbH, Sulzbach, Germany;* [3]*Evonik Degussa GmbH, Hanau, Germany;* [4]*Henkel AG & Co. KGaA, Düsseldorf, Germany;* [5]*Scientific Consultancy – Animal Welfare, Neubiberg, Germany;* [6]*The Chemours Company, Newark, NJ;* [7]*Toxminds BVBA, Brussels, Belgium;* [8]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany;* [9]*Polymer Physics, BASF SE, Ludwigshafen am Rhein, Germany;* [10]*Product Safety, BASF SE, Ludwigshafen am Rhein, Germany;* [11]*Shell Health, Shell International B.V., The Hague, Netherlands;* [12]*Shell Health, Shell Oil Company, Houston, TX and* [13]*Technology and Engineering, AkzoNobel, Arnhem, Netherlands.*

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) proposes Decision-making framework for the grouping and testing of nanomaterials (DF4nano) that consists of 3 tiers to assign NM to 4 main groups and to determine and refine specific information needs. It covers all relevant aspects of a NM life cycle and biological pathways, i.e. material properties and interactions, biopersistence, uptake and biodistribution, cellular and apical toxic effects. Use, release and exposure are used to determine if, e.g. nanomaterials cannot be released from a product matrix. The four main groups encompass (1) soluble NM, (2) biopersistent high aspect ratio nanomaterials, (3) passive nanomaterials, and (4) active nanomaterials. The DF4nano aims to group NM by their specific mode-of-action. This is eventually directed by a NM intrinsic properties. However, since the exact correlation of material properties and apical toxic effect is not yet established,it uses the 'functionality' of nanomaterials for grouping rather than relying on intrinsic material properties alone. Such functionalities include system-dependent material properties, bio-physical interactions, *in vitro* effects and release and exposure. The DF4nano is a tool that applies modern

toxicology and contributes to the sustainable development of NM. It ensures that no studies are performed that do not provide crucial data and therefore saves animals and resources. The The grouping decisions of DF4nano for 24 nanomaterials were validated against grouping by results of existing *in vivo* data.

2545 The Role of Genetic Background on Adverse Health Effects Due to Prenatal Exposure to Environmental Obesogen Tributyltin

J. Tobacyk[1], M. La Merrill[2], S. Luo[1] and A. Harrill[1]. [1]*University of Arkansas for Medical Sciences, Little Rock, AR and* [2]*University of California, Davis, Davis, CA.*

Tributyltin (TBT) is an environmental contaminant that was widely used in industry and agriculture, but its use has been restricted due to adverse endocrine effects on marine life. Recent studies have shown that prenatal TBT exposure at environmentally relevant doses can contribute to adipogenesis and the development of obesity (thus TBT is termed an "obesogen"). In prior studies, TBT altered expression of genes responsible for differentiation of mesenchymal stem cells, driving preferential formation of adipocytes, potentially at the expense of osteoclasts. In this study, the gene by environment effects of prenatal exposure to TBT were investigated using four genetically different mouse strains. Fat and bone formation in prenatally exposed F1 offspring of strains 129S1/SvImJ, C57BL/6J, NOD/ShiLtJ, and PWK/PhJ were quantified at 4 and 9 weeks of age using dual energy x-ray absorptiometry (DEXA). When litter assignments were taken into account, there were no significant effects of TBT observed with regards to % fat, bone mineral density (BMD), or absolute body weight in any strain ($P>0.05$ as compared to F1 offspring of vehicle controls). No steatosis nor adverse histopathology were observed in livers of either vehicle or TBT treated mice as assessed by Oil Red O and hematoxylin & eosin staining. Concordantly, there there was no effect of TBT on glutathione concentrations in the liver tissue (GSH/GSSG), regardless of strain ($P>0.05$), suggesting a lack of oxidative stress. Taken together, the data suggest that *in utero* TBT exposure may not necessarily result in overt effects on absolute fat or bone mass in adolescent or young adult life stages and that the obesogenic potential of TBT may be dependent upon as yet unidentified environmental contexts.

2546 Differential Susceptibility to Diethylstilbestrol Exposure in Genetically Diverse Mice

N. E. Carns, T. Konneker and D. L. Aylor. *Biological Sciences, North Carolina State University, Raleigh, NC.* Sponsor: S. Kelada.

Diethylstilbestrol (DES) is a drug that caused infertility and vaginal cancer in some adults who were exposed prenatally before its use was ended in 1971. Some DES-exposed mouse strains also display reproductive defects (male and female) and cancer; yet other mouse strains are unaffected. This indicates a strong genetic component to susceptibility. Our goal is to identify the gene x environment interactions (GxE) associated with clinical traits and gene expression in DES-exposed inbred mouse strains. We have measured the acute effects of DES exposure on uterine gene expression for a known susceptible strain (FVB/NJ) and a known resistant strain (C57BL/6J). A substantial number of genes are differentially expressed between DES-exposed and control mice in both strains, but only 61% of these genes are shared. This illustrates the large effect genetic background can have on DES-induced changes in uterine gene expression. These strain-specific responses to DES involve coordinate regulation of hundreds of genes and can help narrow down sets of genes associated with disease susceptibility. We have identified gene modules that characterize strain-specific DES response and we relate these to clinical data derived from uterine histopathology. We conclude that the combination of genetic variation with functional genomics is a powerful tool for identifying genes involved in differential susceptibility to toxicants, and we propose that this approach is broadly relevant to other toxicological studies in rodents.

2547 Computational Approach Using Mouse Embryonic Stem Cells to Define a Mechanistic Applicability Domain for Prenatal Developmental Toxicity

S. Hunter[3], M. Rosen[3], N. C. Baker[1], R. Judson[3] and T. Knudsen[3]. [1]*Lockheed-Martin, Research Triangle Park, NC;* [2]*NCCT, US EPA, Research Triangle Park, NC and* [3]*NHEERL, US EPA, Research Triangle Park, NC.*

Identification of putative mechanisms for developmental toxicity was performed by co-analyzing three datasets for the effects of 1065 ToxCast phase Ia and II chemicals: 1. *In vitro* cellular and molecular effects from ToxCast GeneScore; 2. *In vivo* phenotypic effects from prenatal developmental toxicity studies in ToxRefDB; and 3. data from our lab of effects on mouse Embryonic Stem Cell (mESC) differentiation and cytotoxicity at gastrulation (GSTR) or cardiomyocyte (CM) stages. Correlation analysis of ToxCast-Gene associations with *in vivo* and mESC endpoints identified a 'putative mechanism' involving a particular target gene when its gene activity was overrepresented (>2-fold) in the chemicals that produced an apical endpoint. There were 19 *in vivo* developmental phenotypes each associated with 22 to 107 genes. Greater than 60% of the genes associated with jaw hypoplasia, orofacial clefts, and ureteric defects correlated with other phenotypes, whereas fewer than 26% of genes associated with renal, eye, heart, and major vessel defect genes correlated with other phenotypes. A set of 75 genes (including FGFR1, GSK3B, IGF1R, KDR, RXRA) were common to 8 of the 19 phenotypic endpoints. Genes for individual mESC endpoints had a 26% to 59% overlap with *in vivo* genes. Genes of combined mESC GSTR-Cytotox and CM-Differentiation correlated to 75% of all *in vivo*-genes. For example, 87% (27) of the genes associated with *in vivo* heart defects were also associated with mESC including 13 GPCR-pathway genes, 2 CYPs, RXRA, H2AFX, and TP53. This analysis of mESC data reveals a broad range of diverse putative mechanisms that correlated with apical endpoints of prenatal developmental toxicity for ToxCast datasets. Correlation of these genes with known and novel pathways of developmental toxicity are needed; however, this analysis is a starting point for mechanistic prediction in defining the biological applicability domain for prenatal developmental toxicity. This abstract does not represent US EPA policy

2548 Prediction of *In Vivo* Developmental Toxicity of Tebuconazole in Rats Using Physiologically Based Kinetic Modeling-Facilitated Reverse Dosimetry of *In Vitro* Toxicity Data

H. Li[2], M. Zhang[2], J. Vervoort[2], I. M. Rietjens[2], B. van Ravenzwaay[3] and J. Louisse[2]. [1]*Division of Biochemistry, Wageningen University, Wageningen, Netherlands;* [2]*Division of Toxicology, Wageningen University, Wageningen, Netherlands and* [3]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany.*

Toxicological hazard and risk assessment nowadays largely rely on animal testing. For economical and ethical reasons, the development and validation of reliable alternative methods to *in vivo* toxicity testing are urgently needed. *In vitro* toxicity assays provide *in vitro* concentration-response curves, whereas *in vivo* dose-response curves are regularly used for risk assessment. In the present study, we translated *in vitro* concentration-response data for developmental toxicity of an antifungal compound, tebucoanzole, obtained in two alternative assays, i.e. the ES-D3 cell differentiation assay and the *ex ovo* assay of chicken embryos, into *in vivo* doses using a physiologically based kinetic model for rat (Berkeley-Madonna model), that is mainly based on kinetic model parameter values derived using *in vitro* techniques, in a reverse dosimetry approach. The predicted *in vivo* doses were used to derive BMD10 and BMDL10 values for rat, which were then compared with *in vivo* values derived from toxicity data in rats reported in the literature. The results show that the kinetic model based BMD(L)10 values from both assays are in accordance with BMD(L)10 values obtained from *in vivo* data (within 3-fold difference). In conclusion, physiologically based kinetic modeling-facilitated reverse dosimetry of *in vitro* toxicity data is a promising tool to predict *in vivo* dose-response curves and could be therefore used to set a point of departure for deriving safe exposure limits in risk assessment.

2549 *In Vitro*-to-*In Vivo* Extrapolation of Xenoestrogens Using an Estrogen Responsive *In Vitro* Transcriptional Activation Assay and the *In Vivo* Uterotrophic Assay

J. M. Conley[2], B. R. Hannas[2], N. Evans[2], J. Furr[2], V. Sutherland[1], P. M. Foster[1], L. E. Gray[2] and V. S. Wilson[2]. *[1]NTP, NIEHS, Research Triangle Park, NC and [2]ORD/NHEERL/TAD, US EPA, Research Triangle Park, NC.*

Widespread contamination of waters with both natural and synthetic estrogens is a concern for potential adverse ecological and human health effects. *In vitro* assays are valuable screening tools for identifying contaminated environmental samples and chemical specific mechanisms of toxicity, such as estrogen receptor activation. However, *in vitro* effect concentrations cannot necessarily be extrapolated to similar estimates *in vivo* because most *in vitro* assays do not account for toxicokinetics. To explore this issue further, we utilized data from the T47D-KBluc estrogen receptor transactivation assay to predict rat uterotrophic assay response following oral administration of individual chemicals and mixtures. 17β-estradiol (E2), ethinyl-estradiol (EE2), benzyl-butyl phthalate (BBP), bisphenol-A (BPA), bisphenol-AF (BPAF), bisphenol-C (BPC), bisphenol-S (BPS), and methoxychlor (MET) were tested individually, while BPS+MET and BPAF+MET were tested as equipotent mixtures. The observed *in vivo* ED50 values were lower (3 - 1261-fold lower) than those predicted using *in vitro* data for all compounds except BBP, which is metabolically deactivated. Interestingly, BPS (EC50=6.4e-7 M) was less potent *in vitro* than BPA (EC50=1.2e-7 M) by a factor of ~5, however *in vivo* BPS (ED50=150 mg kg-1 d-1) was ~7-fold more potent than BPA (ED50=1118 mg kg-1 d-1). Further, we demonstrated that the uterotrophic response to mixtures of BPAF+MET and BPS+MET could be predicted from the individual *in vivo* data for those compounds using the dose addition model, however predictions using *in vitro* data resulted in ED50 estimates that were 26- and 297-fold greater than observed, respectively. Overall, these data illustrate the potential for underestimating *in vivo* potency from predictions made with *in vitro* data for compounds that undergo significant disposition following oral administration. *In vitro* assays will continue to be valuable for screening, however increasing the complexity of *in vitro* models to account for aspects of toxicokinetics, notably metabolism, and alternative signaling pathways will be necessary to improve the prediction accuracy of *in vitro*-to-*in vivo* extrapolations. Abstract does not reflect US EPA policy.

2550 Bisphenol AF: Correlation of *In Vitro* Endocrine Responses with *In Vivo* Developmental Outcomes (A Case Example)

V. Sutherland[1], K. Pelch[1], B. McIntyre[1], J. M. Conley[4], L. E. Gray[4], J.-H. Hsieh[1], L. Truong[2], R. Tanguay[2], Z. Lundby[3], P. Allard[3] and P. M. Foster[1]. *[1]National Toxicology Program, Research Triangle Park, NC; [2]Oregon State University, Corvallis, OR; [3]University of California, Los Angeles, Los Angeles, CA and [4]US EPA, Research Triangle Park, NC.*

The relationship between *in vivo* animal toxicity testing and alternative assays has not been thoroughly evaluated; therefore, it was of interest to examine the correlations between an *in vivo* multigenerational study and several alternative models for Bisphenol AF (BPAF). BPAF is an analogue of Bisphenol A (BPA) that is used as a crosslinking agent for certain fluoroelastomers and is being evaluated in a number of model systems (e.g. High Throughput Screening (HTS-Tox21), Endocrine Disruptor Tier 1 screening, zebrafish, *C. elegans*, and multigenerational rat studies). BPAF was selected because it has structural similarity to the synthetic estrogen BPA and a lack of adequate toxicity data. HTS-Tox 21 revealed that BPAF exhibited activity at both the estrogen receptor (ER) alpha (full and partial) and androgen receptor (AR). T47D-KBluc transcriptional activation assay indicated that BPAF was a weak ER alpha agonist (EC50 2.06e-8 M) and an antagonist in the CV-1 AR antagonist assay (EC50 1.4e-6 M). Oral administration to rats resulted in a robust uterotrophic response; whereas, administration to Zebrafish and *C. elegans* produced a delay in growth, with death at higher concentrations, or an increase in apoptosis and morphological defects, respectively. In comparison, dietary administration of BPAF at doses up to 3750 ppm in a multigenerational study showed significant functional effects on reproduction and development, including delays in balano-preputial separation (6 and 37 days at the mid and high doses for F1 offspring) and an acceleration in time to vaginal opening (2–10 days for F1 offspring). Similar findings were observed in the F2 offspring. High dose F1 rats also exhibited a low incidence of reproductive tract malformations and were unable to reproduce. A reduction in live litter size and fewer pregnancies were observed at 1125 ppm. Although these alternative assays suggested the potential for biological and weak endocrine activity, the severity of the BPAF-mediated adverse responses observed *in vivo* were unexpected.

2551 Exposure-Based Comparisons of Developmental Toxicity Signals Between Alternative Assays and *In Vivo* Rat with PF-04449913, a Smoothened Inhibitor

C. J. Bowman[1], D. S. Stedman[1], S. Davenport[1], C. Stethem[1], G. Stevens[2] and G. D. Cappon[1]. *[1]Pfizer, Inc, Groton, CT and [2]Pfizer, Inc, La Jolla, CA.*

Alternative developmental toxicity assays have shown value for ranking compounds based on the potential to cause teratogenicity. Expanded use of alternative assays for a weight-of-evidence approach is desirable and could help reduce animal use, but using these assays for risk assessment purposes will require an understanding of the doses and/or exposures at which signals of developmental toxicity are observed across alternative and traditional assays. This study evaluated the developmental toxicity potential of PF-04449913, a Smoothened inhibitor (SMOi), in rat whole embryo culture (rWEC), the zebrafish developmental toxicity assay (ZDTA), the murine embryonic stem cell test (mEST), and an *in vivo* rat study. The rWEC exposed early organogenesis stage rat embryos for 2 days, the ZDTA exposed zebrafish embryos from 6 to 120 hours post fertilization, and the mEST exposed murine embryonic stem cells in differentiation media for 10 days. At the end of exposures, all were scored for evidence of abnormal development/differentiation. The *in vivo* rat study used 8/group with once daily dosing from gestation day 7 to 17 with full fetal external, visceral and skeletal evaluations. Zebrafish tissue and rat plasma were evaluated for PF-04449913 concentrations. PF-04449913 was teratogenic and embryotoxic in rats. All alternative assays predicted PF-04449913 to be teratogenic. There was a dose-dependent increase in WEC and ZDTA phenotype abnormalities and for inhibition of differentiation in the mEST. In the rat study a dose-dependent increase in developmental signals were observed across all dose levels. The lowest concentrations (media or tissue) with evidence of developmental toxicity across the alternative assays ranged from 0.04 µg/mL to 16 µg/mL compared with plasma concentration of 0.7 µg/mL in the rat 4 h post-dose. In summary, for the SMOi inhibitor PF-04449913, this suite of alternative assays adequately predicted teratogenicity and bracketed the lowest observed effect concentration in the rat.

2552a Patient-Specific Stem Cells as Models for Gene, Drug, and Environment Interactions in Disease

A. B. Bowman[1] and J. R. Richardson[2]. *[1]Vanderbilt University Medical Center, Nashville, TN and [2]Department of Pharmaceutical Sciences, Northeast Ohio Medical University, Rootstown, OH.*

With the advent of adult stem cell technology there is an increasing interest in the potential for using stem cells for screening for drug and chemical toxicity and drug efficacy. More important, researchers are now using this technology in an attempt to understand mechanisms behind individual and population differences in these outcomes based on genetics. Indeed, by using a patient's own stem cells and converting them to disease-specific cells, better understanding of disease pathophysiology and, ultimately, a personalized approach for therapeutic intervention may be attainable. Using the powerful tools of genetics and induced pluripotent stem cell (iPSC) technology, the speakers in this symposium will discuss new and cutting-edge research that provides examples of the power of this approach for mechanistic and clinical/translational studies. Speakers representing academia, government, and industry will provide diverse viewpoints on the utilization of iPSC modeling in both broad and specific contexts. The session will begin with a brief 5-minute overview of iPSC and stem cell technology by one of the co-chairs. The first full speaker of the session will utilize several specific examples of how iPSC- based models of genetic disease (diabetes, heart disease, and neurological disorders) have been used to predict drug efficacy and disease-specific vulnerabilities to toxicants. The second speaker will provide a specific example of how iPSC technology can be used to translate pathophysiological mechanisms described in non-human models to this patient-specific human model system (e.g, gene-environment interactions between Huntington's disease and manganese) and utilization of the iPSC model for preclinical testing of small molecules developed in a non-human model system. The third speaker will present how iPSC technology led to discovery of gene-environment interactions in the context of a single common genetic risk factor for Alzheimer's disease. The fourth speaker will discuss how iPSC technology can be leveraged to screen for disease-modifying agents with neuroprotective and/or neurotoxic effects. The final speaker will detail genetic susceptibility to agents that induce carcinogenicity using this technology. By including examples of disease affecting distinct organ systems, this session provides diverse viewpoints of the utility of iPSC technology across disciplines. The format for the presentation will be 25-minute talks, with 4 minutes for audience questions. A 20-minute panel discussion will then address broad applications of the iPSC model system to explore gene x environment x drug interactions in human disease. The panel will address key determinants in successful iPSC-based

model applications, including (a) developmental lineage specificity, (b) cellular/tissue/organ modeling, (c) appropriate controls and sampling size, (d) multigenic versus monogenic effects, (e) statistical concerns, and sample size. Further, the panel will be open to discussions and topics of interest to members of the audience.

S 2552b Use of Human iPSC-Derived Cells as a Means to Investigate the Relationship Between Genes and Disease

B. Anson. *Cardiomyocyte, Cellular Dynamics, Inc, Madison, WI.* Sponsor: A. Bowman.

Induced pluripotent stem cells have changed the fundamental fabric of what is possible in cell biology. From over 60 different diseases, induced pluripotent stem cell (iPSC) derived tissues demonstrate a relevant phenotypic *in vitro* to *in vivo* correlation with pathology. In addition, large banks of iPSC lines from diseased individuals are being created to further connect genetics to phenotypic biology. Further, genetic engineering techniques have improved in efficiency and cost such that isogenetic controls can be made from diseased lines. These iPSC lines enable a better understanding of biological and toxicological consequences of disease and create a means to screen for novel therapeutic modalities. Specifically, iPSC-derived cardiomyocytes from diabetic individuals show a phenotype with the hallmarks of diabetic cardiomyopathy. This model was used to better understand cardiac disease as well as screen libraries for compounds to improve this phenotype. Further, iPSC cardiomyocytes from 250 patients with left ventricular hypertrophy have phenotypic hallmarks of disease that correlate with specific single nucleotide polymorphisms from a larger genome wide association study. This effort, in collaboration with NHLBI and the Medical College of Wisconsin, was one of the largest efforts to demonstrate the functional impact of genetic diversity. This presentation will focus on toxicological utility of iPSC derived tissues using exemplary cases including genetics of diabetes, left ventricular hypertrophy, and Central Nervous System (CNS) diseases.

S 2553 Patient-Derived iPSC Models as a Translational Tool Between Human and Non-Human Model Systems for Environmental Health Research

A. B. Bowman. *Vanderbilt University Medical Center, Nashville, TN.*

Induced pluripotent stem cells have changed the fundamental fabric of what is possible in cell biology. From over 60 different diseases, induced pluripotent stem cell (iPSC) derived tissues demonstrate a relevant phenotypic *in vitro* to *in vivo* correlation with pathology. In addition, large banks of iPSC lines from diseased individuals are being created to further connect genetics to phenotypic biology. Further, genetic engineering techniques have improved in efficiency and cost such that isogenetic controls can be made from diseased lines. These iPSC lines enable a better understanding of biological and toxicological consequences of disease and create a means to screen for novel therapeutic modalities. Specifically, iPSC-derived cardiomyocytes from diabetic individuals show a phenotype with the hallmarks of diabetic cardiomyopathy. This model was used to better understand cardiac disease as well as screen libraries for compounds to improve this phenotype. Further, iPSC cardiomyocytes from 250 patients with left ventricular hypertrophy have phenotypic hallmarks of disease that correlate with specific single nucleotide polymorphisms from a larger genome wide association study. This effort, in collaboration with NHLBI and the Medical College of Wisconsin, was one of the largest efforts to demonstrate the functional impact of genetic diversity. This presentation will focus on toxicological utility of iPSC derived tissues using exemplary cases including genetics of diabetes, left ventricular hypertrophy, and Central Nervous System (CNS) diseases.

S 2554 Modeling Gene-Environment Interactions in Alzheimer's Disease

J. R. Richardson. *Department of Pharmaceutical Sciences, Northeast Ohio Medical University, Rootstown, OH.*

Using well-characterized clinical cohorts, we recently reported that serum levels of DDE, the metabolite of the organochlorine pesticide DDT is significantly higher in the serum of Alzheimer's Disease (AD) cases. Serum DDE levels were also associated with increased risk of AD diagnosis. Further, individuals harboring a polymorphism in APOE and having high serum levels of DDE performed worse on a cognitive task than either those with just the polymorphism or high DDE levels. Studying gene-environmental interactions from a mechanistic standpoint is often hampered by the necessity of expressing altered genes in heterologous systems. One emerging way to deal with this issue is through the use of stem cells. It is not possible to make human embryonic stem cells (hESCs) from individuals affected with AD since they are not diagnosed until late in life. The discovery that adult mouse or human fibroblasts could be reprogrammed to "induced pluripotency" provided a novel opportunity for modeling human neurons based on genotypes with known disease histories. We are currently using several model systems, including isogenic cell lines stably expressing human APOE isoforms and induced pluripotent stem cells (iPSCs) from individuals representing different APOE genotypes, to determine potential mechanisms responsible for the worsened cognitive function in APOE4 individuals with high serum DDE levels. To date, we have identified that DDT and DDE increase levels of amyloid precursor protein (APP) and A_ secretion, both of which appear to be exacerbated in the presence of the APOE4 genotype.

S 2555 Leveraging Novel Technologies for Human iPSC-Based Screening

X. Zeng. *XCell Science Inc., Novato, CA.* Sponsor: A. Bowman.

Human induced pluripotent stem cell (iPSC) technology offers the benefits of a cell line coupled with the advantage of using human primary cells. For example, iPSC can be obtained in large numbers and be differentiated into multiple cells type allowing screening in the same allelic background. Moreover, multiple iPSC lines can be generated to permit sampling genetic diversity, and iPSC lines can be genetically engineered to allow generation of assay ready cells. Finally, human disease mutations can be captured in a stable cell population and isogenic controls for human disease can be readily generated. We tested this possibility with neural lineage for neurodegenerative disorders. Our approach includes: 1) making a panel of lines, 2) generating differentiated neural cells in assayable format, and 3) running demonstration screens. Specifically, we have developed a large panel of genetically engineered human iPSC lines including 1) control lines, 2) lines from patients with mono allelic disease, 3) knock-in reporter lines, 4) isogenic controls of single and double knock-outs. Equally importantly, we are able to produce large numbers of differentiated cells including neural stem cells, neurons and astrocytes in an assay ready format from these engineered iPSC lines, and run demonstration screens for neuroprotective and neurotoxic effects. I will illustrate this approach by a Parkinson's Disease (PD) panel that identified compounds with neuroprotective effects including rasagiline and selegiline.

S 2556 Differential Susceptibility of Stem Cells in Inorganic Carcinogenesis

E. Tokar. *National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

We have used human adult stem cell (SC) models to demonstrate the differential effects exerted by arsenic and cadmium, two common environmental carcinogens, during the formation of cancer stem-like cells (CSCs). Stem cells showed an innate resistance and hyper-adaptability to arsenic which was in direct contrast to the initial high sensitivity of the SCs to cadmium. Opposing effects between the inorganics were due to differential regulation of factors involved in apoptosis (e.g. BCL2; BAX; Caspases 3, 8, 9), metal uptake or efflux (e.g. ABCC1; ABCC2; ZIP8; ZnT1), and metal resistance or adaptability (e.g. NRF2; metallothionein; MTF-1; GST-pi) during the formation of CSCs. Arsenic and cadmium also had contrasting effects on key genes and signaling pathways involved in differentiation and organogenesis (e.g. FGFs, orphan nuclear receptors, Notch, etc.). This observed resistance of SCs to arsenic and resultant CSC overabundance in arsenic cancers, together with the initial sensitivity of SCs to cadmium, followed by the rapid recovery of these cells as a highly aggressive CSC-like phenotype, indicate major differences between these two inorganic carcinogens. Together, these data help explain the role of SCs/CSCs and mechanisms involved in the carcinogenesis of these two inorganics.

2557a Sulfur Mustard Poisoning: Mechanisms of Dermal and Pulmonary Toxicity and New Treatment Approaches

A. Vale[1] and H. Thiermann[2]. [1]*University of Birmingham, Birmingham, United Kingdom and* [2]*Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany.*

Sulfur mustard (Bis[2-chloroethyl] sulphide, SM), a chemical sometimes referred to as mustard gas, is a liquid that boils at 217C and freezes at 13C to 15C, which explains its persistence in the environment. Droplets of SM released in an explosion can pose a risk to health from inhalation, ingestion of contaminated food and water, as well as contact with the skin and eyes. SM was first used as a chemical warfare agent almost 100 years ago (July 12, 1917) in Belgium in WWI. Since then it has been deployed in Ethiopia, China, Yemen, and Iran with several hundred thousand casualties resulting. The potential further use of SM in military conflicts and by terrorists remains a threat that if realized would result in a large number of casualties with severely incapacitating eye, skin, respiratory tract, and possibly systemic damage. SM produces acute damage to the skin (blisters, skin necrosis), to the eyes (corneal damage with temporary blindness), and to the respiratory tract (nose bleeds, tracheobronchitis, and acute respiratory distress syndrome), and it can be lethal at high concentrations. SM depresses bone marrow function, which may lead to secondary infection. Long-term disability due to respiratory complications is common. SM is also a recognized human carcinogen. Following SM inhalation, DNA damage, apoptosis, and autophagy are observed in the lung, along with increased expression of activated caspases and DNA repair enzymes, biochemical markers of these activities. This is associated with inflammatory cell accumulation in the respiratory tract and increased expression of tumor necrosis factor-a and other pro-inflammatory cytokines, as well as reactive oxygen and nitrogen species. Matrix metalloproteinases are also upregulated in the lung and skin after SM exposure, which are thought to contribute to the detachment of epithelial cells from basement membranes and disruption of the pulmonary epithelial barrier. Findings that production of inflammatory mediators correlates directly with altered lung function suggests that they play a key role in toxicity. Following skin contact with SM, keratinocytes of the stratum basale in the skin appear to be the most sensitive to its cytotoxic actions, and blistering involves the detachment of these cells from the supporting basal lamina of the basement membrane of the epidermal-derma junction. Studies on SM and its analogs in animals suggest that individual or combination therapies using anti-inflammatory agents (e.g., steroids), antioxidants (e.g., tocopherols, melatonin, acetylcysteine, nitric oxide synthase inhibitors), and protease inhibitors (e.g., doxycycline, aprotinin, ilomastat), may be effective in ameliorating the toxicity of SM-induced lung damage in humans. Experimentally, single and multiple infusions of mesenchymal stem cells have reduced SM-induced edema, necrosis, inflammation, and death at 5 weeks post-exposure. In addition to symptomatic and supportive treatment for SM-induced blisters, mechanical dermabrasion and laser debridement (lasablation) have both produced an increased rate of wound healing in animal models and may be of benefit in a clinical context. A rapid test using an antibody for the reliable detection of pure SM on the skin, which gives a result within 10 minutes, has been developed.

2557b Sulfur Mustard: History of Use and Features of Exposure

A. Vale. *School of Biosciences, University of Birmingham, Birmingham, United Kingdom.*

Sulfur mustard (Bis(2-chloroethyl) sulphide; SM), a chemical sometimes referred to as mustard gas, is in fact a liquid which boils at 217°C and freezes at 13°C to 15°C, which explains its persistence in the environment. Droplets of SM released in an explosion can deposit on numerous surfaces, evaporating slowly and posing a risk from inhalation, ingestion of contaminated food and water, as well as contact with the skin and eyes. SM was first used as a chemical warfare agent on the night of 12 July 1917 at Ypres, Belgium. By the end of WWI there were more than 120,000 British casualties. Italian forces used SM against Ethiopian forces in 1936, it was used by the Japanese against Chinese troops in WWII, and by Egyptian forces in Yemen (1963-7). Iraq used SM against Iranian soldiers in the Iran-Iraq war (1983-1988) and more than 100,000 casualties resulted from some 380 chemical attacks. SM produces damage to the skin (blisters, skin necrosis), to the eyes (corneal damage with temporary blindness) and to the respiratory tract (nose bleeds, tracheobronchitis and acute respiratory distress syndrome), and it can be lethal at high concentrations. SM also depresses bone marrow function which may lead to secondary infection. Long-term disability due to respiratory complications is common. SM is also a recognized human carcinogen. The potential further use of SM in military conflicts and by terrorists remains a threat that if realised would result in a large number of casualties with severely incapacitating eye, skin, respiratory tract, and possibly systemic, damage.

2558 Pathophysiology of Sulfur Mustard-Induced Skin Lesions and Current Therapeutic Options

P. Rice. *CBR Division, Dstl Porton Down, Salisbury, United Kingdom.* Sponsor: A. Vale.

Although SM is a relatively nonselective alkylating agent, keratinocytes of the stratum basale appear to be the most sensitive to its cytotoxic actions and blistering involves the detachment of these cells from the basement membrane of the epidermal-dermal junction. Clinically, erythema develops within a few hours of exposure. Vesication usually begins on the second day after exposure and may progress for up to two weeks. Necrosis of the epidermis and superficial dermis is complete 4–6 days after exposure and separation of necrotic slough then begins. Scab formation begins within 7 days. By 16–20 days, separation of slough is complete and re-epithelialization begins. Wound healing is considerably slower than that for a comparable thermal burn (complete healing may take 3–8 weeks) and patients often require extended hospital treatment. The development and healing of SM-induced skin burns has not only been observed in human casualties but has been studied recently at the gross, microscopic and ultrastructural levels in several animal models. The current clinical management strategy for such burns is essentially symptomatic and supportive, following similar lines to that for thermal burns. Recently, two techniques for re-surfacing the burn and improving the rate of wound healing have been investigated. Mechanical dermabrasion and laser debridement ("lasablation") both produced an increased rate of wound healing in animal models and may be of benefit in a clinical context.

2559 Sulfur Mustard-Induced Toxic Lung Injury: Mechanisms of Toxicity

A. Sciuto. *Research Division, US Army Medical Research Institute of Che, Aberdeen, MD.*

More than half of surviving sulfur mustard (SM)-exposed casualties in the Iran/Iraq conflict suffer from permanent lung injuries. The mechanisms and timing of the development of these pathologies are poorly defined, and there is no effective antidote. Rats were intubated and ventilated for 10 min with nebulized HD or vehicle to achieve doses of 0, 0.5, 1.75, 2.25, and 3 mg/kg. Pulmonary function was analyzed by whole-body plethysmography. Rats were euthanized at various time-points = 6 months post-exposure, blood chemistry was analyzed, broncho-alveolar lavage fluid was analyzed for cytokines/ redox state, and lungs were subjected to pathologic analysis. The data show high correlation between blood gas perturbations, upper airway necrosis and pulmonary function in the 24 h to 48 h after the 3 mg/kg HD exposure. In longer-ranging animals, the 3 to 7 weeks period was a significant challenge because 15% of the 3 mg/kg group and 10% of the 2.25 mg/kg group died suddenly or required withdrawal from the study during this time. Alveolar exudates, edema, oxidative stress, and inflammation peaked at 3 weeks and correlated with changes in pulmonary function and respiratory distress. Therefore, the first day and the second to third weeks post-exposure may be crucial windows in the progression of HD inhalation injury, and treatment at these times may be the best approach. Based on this, we have evaluated single and multiple infusions of mesenchymal stem cells as a treatment and have found success in reducing SM-induced edema, necrosis, inflammation, and death at 5 weeks post-exposure.

2560 Sulfur Mustard-Induced Pulmonary Injury: Current Therapeutic Approaches to Mitigating Toxicity

D. Laskin. *Department of Pharmacology & Toxicology Rutgers University, Piscataway, NJ.*

Sulfur mustard (SM) is highly toxic to the lung inducing both acute and chronic effects including upper and lower obstructive disease, airway inflammation, and acute respiratory distress syndrome (ARDS), and with time, tracheobronchial stenosis, bronchitis, and bronchiolitis obliterans. Thus, it is essential to identify efficacious treatments for both acute and chronic lung diseases induced by this vesicant. Studies on SM and its analogs in animals suggest that individual or combination therapies using anti-inflammatory agents (e.g., steroids), antioxidants (e.g., tocopherols, melatonin, acetylcysteine, nitric oxide synthase inhibitors), and protease inhibitors (e.g., doxycycline, aprotinin, ilomastat), may be effective in ameliorating the toxicity of SM in humans. However, use of these countermeasures is limited due to their relatively non-specific actions. Consequently, there remains a pressing need to identify more specific therapeutics, and effective strategies for delivering these agents to target organs. Lung injury in ARDS patients is reduced by conventional mechanical ventilation, sedation, and decreased threshold for blood cell transfusion. Other promising therapeutic approaches to ARDS include

endotracheal surfactant, high-frequency oscillatory ventilation, noninvasive ventilation, extracorporeal membrane oxygenation, corticosteroids, and restrictive fluid management. For chronic injury associated with COPD, inhaled anticholinergic and ß-agonist bronchodilators are useful in ameliorating symptoms, and corticosteroids have some limited utility in reducing inflammation associated with severe disease. In order to devise a uniform and rational approach to treating SM toxicity that minimizes untoward effects, it is essential to integrate the knowledge gained from cell culture and animal studies, human exposure, and current clinical management of acute and chronic lung injury.

S 2561 Novel Developments and Advanced Molecular Targets for Diagnosis and Treatment of Sulfur Mustard-Induced Cell Damage

H. Thiermann. *Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany.*

Exposure to sulfur mustard (SM) results in the alkylation of DNA-adducts at guanine and adenine bases. The N7-guanine mono-adduct, that accounts for up to 60% of all observed DNA-alkylations, can be visualized using a specific antibody by slot-blot and immunocytochemistry techniques. In general, these procedures are time consuming and require advanced technical equipment. To overcome this problem in a mass casualty scenario, we have developed a rapid test using an antibody for the reliable detection of pure SM on the skin, which gives a result within 10 minutes. Currently, an enhanced on-site test for the detection of DNA-bound SM in tissues is under development. Also promising is the development of SM resistant skin cells for better understanding the mechanisms of SM-induced cytotoxic damage. SM is a highly reactive compound that can result in severe damage to the skin, lungs and eyes. Although mortality is low (2-3 %), the majority of the fatalities that occur after SM exposure follow inhalation and are caused by SM-induced toxic lung injury. Until recently this was considered as a non-specific damaging process. However, it has now been demonstrated that pulmonary chemosensing TRP-channels are activated by alkylating agents. Overexpression of TRPA1 channels especially results in amplification of SM toxicity which can be rescued by TRP-channel inhibitors. Thus, inhibitors of specific TRP-channels are promising candidate compounds in counteracting SM-induced pulmonary toxicity. Another treatment approach is to stimulate the body's own healing process by influencing stem cell migration, a process overridden after SM exposure.

W 2562a An Update on Juvenile Animal Testing

G. F. Weinbauer[1] and T. P. Coogan[2]. [1]*Covance, Muenster, Germany and* [2]*Janssen Research & Development, LLC, Spring House, PA.*

In recent years, consideration of whether and when relative to clinical plans juvenile animal (JA) testing is needed to support the clinical development of a pharmaceutical has become an important part of the drug development portfolio. An increasing number of these studies are being performed or are planned. The purpose of this scientific session is to present the current scientific and regulatory environment focusing on the "hows" and "whats" of JA testing today, specifically, the perspectives of the pharmaceutical industry, including strategies both internal and through regulatory interaction. In terms of animal models used, these range from rodents to nonhuman primates, with a variety of different study design approaches to evaluate toxicity in JA. Real and hypothetical case study examples are being used to highlight when testing was considered necessary and to illustrate strategies and experiences. Five recognized topical experts will present their perspectives on JA testing: the first presentation provides a state-of-the art overview and update on the ICH S11 (Nonclinical Safety Testing in Support of Development of Pediatric Medicines) status; the second presentation focuses on surprises encountered during JA study conduct; the third presentation highlights the importance of using an integrated approach and involving the toxicologist early in the drug development program; the fourth presentation addresses the impact of different strategies and JA study designs; and the fifth presentation provides a US regulatory perspective and a review of the US FDA study database.

W 2562b Juvenile Animal Testing Today

P. Baldrick. *Covance Laboratories Ltd, Harrogate, United Kingdom.* Sponsor: G. Weinbauer.

Juvenile Animal (JA) testing can now be a part of the modern nonclinical data package to support drug development. However, as a more recent regulated requirement, the "how's" and "what's" of JA testing strategy are still evolving. This presentation highlights why we are where we are today and explains why JA testing has different considerations compared to adult animal studies based around functional and kinetic differences. Currently available information on submitted pediatric investigations plans (PIPs) and associated experiences with regulatory agencies from a European perspective have been reviewed. Available information shows that nearly 200 PIP decisions had juvenile animal work requested, largely involving toxicology testing in the rat and commonly in the oncology, cardiovascular diseases, infectious diseases and endocrinology therapeutic areas. The presentation will discuss current testing paradigms which often involve standard toxicology study designs and comment on considerations to move to more focused tested to enhance the utility of JA testing. Finally, the question of whether studies in juvenile animals are identifying new findings in an organ system not previously seen in adult animals that could be termed "novel toxicity" vs findings solely related to maturation or higher drug exposure differences will be addressed as well as considerations for the future of JA testing and a status update on the planned new pediatric guideline ICH S11.

W 2563 Expect the Unexpected–Learning from the Past

G. P. Bailey. *Janssen Pharmaceutica N.V., Beerse, Belgium.* Sponsor: G. Weinbauer.

Harmonization of the preclinical studies for pediatric development may present us with a more understandable and usable framework within which to design our tailored studies. This framework however, is only possible because of the accumulated knowledge from those "brave pioneers" who pushed the science forward without today's clearer understanding of the issues and designing studies that encompassed all the areas of investigation that were felt to be important for the new drug. It is without surprise of course that things didn't always go to plan with unexplained and unexpected toxicity in all groups including the controls at specific days of age; or they were presented with unusual situations which required elegant designs, lateral thinking and development of new information on age-related development such as the ontogeny of CYP enzymes and transporters. Despite these challenges many innovative designs and new techniques were developed (e.g. microsampling for toxicokinetics), which have subsequently shaped current attitudes as to the way forward for the science. Despite the many pitfalls that were encountered and advances that have occurred in the last few years this does not mean that we cannot still learn from these earlier examples. This presentation will use actual case studies to illustrate the significant issues arising from CYP ontogeny where marked differences in exposure (75x higher brain exposure in a 19 day old rat compared with adult) and the changes observed over a short development period (100 fold lower NOAEL at 10 days than at 21 days of age) and solutions for problems with tolerability using separate cohorts, with cyclical dosing and dosing during the sensitive period only that were developed in this relatively new area of research.

W 2564 Beyond Juvenile Animal Toxicity Studies: The Toxicologist's Broader Role in Pediatric Drug Development Plans

L. L. Morford. *Eli Lilly and Company, Indianapolis, IN.*

Pediatric drug development plans are now required for nearly every compound and geographical region. The timing of discussions of these pediatric plans with regulatory agencies generally vary across region but, in general, these plans must be fairly detailed prior to significant investment in the compound. This need to assess the need for, and design of, juvenile toxicity studies fairly early in the overall drug development program presents a number of challenges. For example, many of the potential pediatric conditions are not necessarily the same as the intended adult indication, resulting in the need for a thorough assessment earlier in the planning cycle. In addition, there may be many potential pediatric indications for a compound and these indications may not include the same patient populations and/or areas of concern. Thus, the nonclinical support of the pediatric plan is dependent upon a number of factors, including formulations, clinical plans and regulatory submission strategies and cannot be developed in isolation. And, just as these other areas play an important role in developing the nonclinical strategy, the juvenile toxicity study may impact these other areas as well, potentially impacting timing and labeling. Therefore, the toxicologist plays an important

role in developing and implementing the overall pediatric plan. This presentation reviews realistic scenarios to help the toxicologist proactively identify potential challenges to the overall pediatric plan as part of a team based approach with less emphasis on the design and conduct of the juvenile toxicity studies per se.

W 2565 Choose Your Own Adventure: Designing Juvenile Animal Toxicity Studies

C. J. Bowman. *Pfizer Inc, Groton, CT.*

There is a desire for regulatory harmonization of what nonclinical toxicity studies are useful to support pediatric drug development. One of the challenges is that there is no standard JA toxicity study design that will appropriately support the multitude of different pediatric populations while building on the theoretical toxicological concerns based on pharmacology and previous data. Rather, it is appropriate to evaluation existing nonclinical and clinical information to address potential differences in sensitivity and reversibility of effects, as well as evaluate theoretical concerns that cannot be adequately or safely tested in pediatric trials by designing JA toxicity studies that target those potential differences and/or concerns in specific pediatric patient populations. To date, different nonclinical strategies have been used to support pediatric drug development ranging from 1-month repeat-dose general toxicity studies in young rats to targeted case-by-case study designs based on known toxicities and a specific pediatric patient population. This presentation uses hypothetical, but realistic, pediatric scenarios with different nonclinical strategies and study designs in the context of global regulatory dialogue and changing clinical development plans. Different strategies and study designs within each scenario will illustrate advantages and disadvantages thus defining important considerations that include studies in multiple species, general toxicity endpoints, requested changes in the clinical plan, biologics vs small molecules, different indications, and impact of regulatory strategy and feedback. Despite no one-size-fits-all approach, careful consideration to product and patient-specific characteristics should provide justification for appropriately designed JA studies when deemed necessary.

W 2566 Regulatory Perspectives on the Role of Juvenile Animal Studies in Global Pediatric Product Development

T. McGovern. *US FDA, CDER, Office of New Drugs, Silver Spring, MD.*

Juvenile animal studies are often conducted when nonclinical data are insufficient to support initiation of pediatric clinical trials in different age groups. At present conduct of juvenile animal testing is determined on a case-by-case basis. There is concern that the approach for juvenile animal testing varies across regions and industry. This talk will provide information on juvenile animal studies in pediatric drug development focusing on the value, design, and criteria for determining the need to conduct these studies. Evaluation of data from an ongoing data mining of the FDA's electronic data submission system and FDA reviews covering the juvenile animal studies that were requested and/or submitted to the Agency will be discussed. Evaluation of how the data were used in pediatric development programs, in regulatory decisions and in the labeling will be addressed. For example, in one case, a study in rats identified a unique toxicity (neurobehavioral and neurohistopathological) with a possible clinical correlate; the information was included in the product label. In another case, a study in dogs identified unexpected cardiac effects that resulted in a clinical hold on pediatric clinical trials until a directed follow-up study was conducted to further characterize the findings. In other cases, findings from juvenile animal studies resulted in Postmarketing Requirements for clinical and nonclinical studies.

W 2567 Moving Beyond Cancer: Current State of the Science of Noncancer Health Effects of Arsenic

D. J. Carlin[1] and J. S. Lee[2]. [1]*NIEHS, Research Triangle Park, NC and* [2]*US EPA, Research Triangle Park, NC.*

Inorganic arsenic (iAs) contamination from geologic, anthropogenic, and food origins is an increasing concern for the US and globally. This metalloid is associated with cancers of the bladder, kidney, liver, prostate, skin, lungs, and nasal cavity, as well as noncancer health effects. Moreover, other considerations in studying cancer and noncancer effects include prenatal exposure to iAs, individual variation in arsenic metabolism efficiency, bioavailability, transport, and speciation. This workshop will highlight the current state of research of iAs and its role in noncancer health effects. Discussions will focus on the impact of iAs exposure on cardiovascular disease, increased susceptibility to infectious diseases, alterations on lung development, and interference with reproduction. This workshop will also begin to address the substantial research gaps regarding the mode of action (MOA) by which iAs could induce or exacerbate these health effects. The US EPA Integrated Risk Information System (IRIS) Program is currently developing a new iAs assessment that considers both cancer and noncancer effects from oral, inhalation, and dermal routes of exposure. Discussion will include current research efforts from epidemiological, animal, and *in vitro* studies and will focus on noncancer health effects that are under consideration by the IRIS Program.

W 2568 Cardiovascular Outcomes Associated with Low-to-Moderate-Dose Arsenic Exposure in Bangladesh

M. Argos. *University of Illinois at Chicago, Chicago, IL.* Sponsor: D.J. Carlin.

Chronic arsenic exposure through drinking water has become a growing public health issue affecting millions of people worldwide, including 35 to 77 million in Bangladesh. Arsenic exposure has been associated with cardiovascular disease and hypertension at moderate- to high-doses, although epidemiologic evidence at low-dose exposure levels (<100 µg/L) is still limited. As part of the Columbia University Superfund Research Program, we established in 2001 the Health Effects of Arsenic Longitudinal Study (HEALS)—a large prospective cohort study based on individual-level data among a population exposed to a wide range of inorganic arsenic from drinking water in Araihazar, Bangladesh. More than 20,000 men and women have been recruited (with >97% response rates) and detailed questionnaires, clinical data, and biospecimen samples have been collected from them at baseline and biennially thereafter. We have prospectively evaluated the effects of various measures of arsenic exposure, including effects at the low-dose range, on incidence and mortality from cardiovascular diseases. Significant dose-response association were observed for all CVD (hazard ratio (HR)=1.15; 95% CI: 1.01, 1.30) and heart disease (HR=1.20; 95% CI: 1.04, 1.38) incidence with a 1-SD increase in baseline water arsenic. These findings will be discussed with an emphasis on the low-dose exposure range.

W 2569 Arsenic and Pulmonary Infections

B. Stanton. *Dartmouth College, Hanover, NH.*

Exposure to high levels of arsenic in Asia and South America has been linked with a variety of inflammatory lung diseases including pneumonia, COPD, bronchiectasis, and chronic bronchitis, all of which are accompanied by bacterial infection. Although it has been shown that low-level exposure to arsenic (2 µg/L, well below the EPA limit of 10 µg/L) in zebrafish and mice alters the immune response to viral and bacterial pathogens, nothing is known about effects of low levels of arsenic on the innate immune function of human lung in response to bacterial infections. Accordingly, the goal of our research is to examine the impact of low levels of arsenic and methylated metabolites of arsenic on *Pseudomonas aeruginosa* induced cytokine secretion in human bronchial epithelial cells (HBEC) and macrophages. iAs, MMA and DMA alone (2-10 µg/L) had adverse effects on the secretion of proinflammatory cytokines stimulated by *Pseudomonas aeruginosa*, as did a combination of iAs, MMA and DMA (2-10 µg/L). These studies demonstrate, for the first time, that exposure to low levels of arsenic, that are relevant to the US population, have adverse effects on cytokine secretion by HBEC and macrophages, which will lead to reduced lung function.

W 2570 Health Effects of Early-Life Inhalation Exposure to Arsenic-Containing Dusts

R. C. Lantz. *University of Arizona, Tucson, AZ.*

Exposure to arsenicals in dust and aerosols generated by mining, mineral processing and metallurgical extraction industries is a serious threat to human populations worldwide. Individuals exposed during sensitive developmental times may be at increased risk from exposure to arsenic containing dusts. Mice exposed by inhalation to arsenic containing dusts during *in utero* and/or postnatal development developed alterations in airway epithelial barrier function, alterations in extracellular matrix around airways and increased airway reactivity. These phenotypic changes are indicative of altered epithelial to mesenchymal transition (EMT). EMT pathways affected most by the exposures was dependent on the developmental age. Alterations in SNAIL family genes were most prominent in 12 day old animals while alterations of ZEB family

genes were more prominent in 28 day old animals. This may be indicative of a progression from partial EMT (12 day old) to the mesenchymal state (28 day old). Transforming growth factor beta, a master regulator of EMT, was also upregulated by arsenic exposure during postnatal lung development. Concomitant with the increased gene expression, EMT protein levels were increased in airway epithelium. Arsenic exposure during vulnerable developmental times can alter normal developmental EMT signaling, resulting in altered pulmonary structure and function.

W 2571 The Impacts of Low-Moderate-Level Drinking Water Arsenic Exposure on Reproduction

M. S. Bloom. *University at Albany, State University of New York, Rensselaer, NY*. Sponsor: D.J. Carlin.

This presentation will cover the reproductive health impacts of groundwater arsenic contamination. Areas with low-moderate levels of groundwater arsenic contamination (<10 µg/L) are widespread yet there are few data to assess risks. To begin to address this data gap, we initiated a case-control pilot study in an area of Romania known for groundwater arsenic contamination. Women with ongoing pregnancies (n=150) or with spontaneous pregnancy losses (n=150) completed questionnaires and provided urine and blood specimens, and we collected residential drinking water samples for arsenic analysis employing hydride generation-atomic absorption spectrometry. Arsenic ranged from undetectable to 130.30 µg/L, with 90% <9.39 µg/L. Assessing gestational exposure with questionnaire data weighted by water arsenic levels, the odds for pregnancy loss were 75% higher among women with co-exposure to cigarette smoke and arsenic than for unexposed women (P=0.057). Among prospectively-followed control women with a live singleton delivery (n=122), gestational co-exposure to cigarette smoke and arsenic was associated with -2.45 (P=0.018) and -1.17 (P=0.029) lower birth weight and birth length Z-scores, respectively, compared to unexposed women. Given that cigarette smoking remains common, especially in low to middle income countries, and that low-moderate level groundwater arsenic contamination is widespread, a more definitive future, biomarker-based investigation is merited.

W 2572 Current Status of the IRIS Arsenic Health Assessment

J. S. Lee. *US EPA, Research Triangle Park, NC.*

The US Environmental Protection Agency (EPA) is developing a state-of-the-science toxicological review on inorganic arsenic for the Integrated Risk Information System (IRIS) Program. In 2011, Congress directed EPA to develop a new inorganic arsenic assessment of cancer and noncancer health effects to be reviewed by the National Academies National Research Council (NRC). Based on recommendations from stakeholders and congressional mandate, the toxicological review will examine the cancer and noncancer effects from oral, inhalation, and dermal inorganic arsenic exposure. At the direction of the NRC, the EPA is (1) incorporating a detailed systematic review of the database to inform study selection for hazard identification and dose-response, (2) conducting mode of action analyses for a number of effects to potentially inform dose-response model selection in the event that epidemiological data are inadequate to characterize dose-response at lower U.S. exposure levels, (3) focusing on characterizing the low dose region of the dose-response relationship (< 100 ug/L drinking water or equivalent concentrations) using meta-analyses of pooled data sets and multiple dose-response models, (4) considering covariates such as smoking, (5) evaluating the additional risks that may be experienced by sensitive subpopulations particularly unborn children and the very young, and (6) conducting probabilistic uncertainty and sensitivity analyses to better inform risk managers. These features are intended to address unresolved uncertainties associated with conventional approaches to the assessment of arsenic. This talk will present innovative aspects of the assessment related to the evaluation of hazard and dose-response for noncancer health effects.

W 2573a Paradigm Change in Toxicology: What Will It Take to Bring Advances in the Science of Toxicology into Regulatory Use?

K. Tsaioun[1] and J.-M. Sauer[2]. [1]*Safer Medicines Trust, Cambridge, MA and* [2]*Critical Path Institute, Tucson, AZ.*

Most experts agree that current test methods for prediction of hazardous effects are not always adequate to ensure the safety of human subjects/consumers exposed to medicines and other chemicals. Major advancements in the science of mechanistic toxicology have produced a number of technologies that can predict various mechanisms of human toxicity. Although academic groups, large industry users and spun-out companies have made major headway in generating data in promising *in vitro* models, resulting in significant use of the technologies in discovery stages, less progress has been made toward qualification/validation of these models for regulatory use. Until consensus is achieved between all stakeholders on the needed level of qualification/validation, global regulatory authorities will be slow to accept the new methods. Old methods will continue to be required by global regulatory authorities if these technologies are not universally accepted. Hence, there is a missing step between the discovery/lead optimization stage and regulatory and commercial use of these technologies. To address this problem, we have assembled a group of academic and industry experts, and brought them together with nonprofit organizations and regulatory authorities to assess the current state of the field and discuss acceptable paths forward in the adoption of new methods. Validation and case studies of practical use in pharmaceutical, industrial chemicals, and cosmetic industries will be presented, and a panel of experts will offer their opinion on which bodies may be best suited to perform the task of standardization of the adoption process, how this process should be implemented, how these new types of data are incorporated into the assessment process, and how regulators assess these data in replacement of traditional *in vivo* studies to give product approvals. A discussion on key drivers for this process will follow stressing similarities and distinctions between pharmaceuticals, cosmetic, agrochemicals, and biocide safety assessments. A working group of the key stakeholders will be formed with the objective of defining agreed-upon criteria for inclusion of alternative tests into regulatory batteries for different industries.

W 2573b REACH and Predictive Non-Animal Approaches

D. Knight. *European Chemicals Agency, Helsinki, Finland*. Sponsor: K. Tsaioun.

The REACH Regulation has various options on how non-animal approaches could be used to inform on (eco)toxicological properties of substances. There are obligations on registrants to consider use of alternatives to vertebrate animal tests where these would be adequate to meet the (standard) information requirements. REACH information requirements increase with increasing tonnage levels, and that the information requirements describe the information which is required and are not per se requirements for animal studies. This means that it may be possible to cover any specific information requirement with adequate alternative methods. The General 'adaptation' rules in Annex XI of the REACH Regulation describe these possibilities. These include the use of: existing non-GLP and non-guideline data; historical human data; weight of evidence (WoE); (Q)SAR predictions; *in vitro* methods; grouping of substances and read across (RE); and substance-tailored exposure-driven testing. A statistical analysis of registrations by ECHA shows that registrants make often make use of the possibilities of Annex XI.
Requirements for acceptance are described at Annex XI and in ECHA Guidance. In general, the main principles are that information from use of alternatives is equivalent to that of the (standard) information requirement, the results are adequate for the purpose of classification and labelling and/or risk assessment, documentation is adequate and reliable, and, depending on the nature of the approach, the scientific validity of the methods has been established and the applicability domain is appropriate. A combination of various methods in a WoE or read-across adaptation could provide adequately high confidence to the prediction and these approaches are encouraged to be developed.

W 2574 CDER Acceptance of Alternative Assays

A. Jacobs. *US FDA, Washington, DC.*

Alternative assay development is an on-going exercise that has the potential to reduce animal use. Depending on the question being addressed, alternative assays can be accepted for regulatory use, if they are scientifically valid. The alternatives usually don't need to undergo formal validation/qualification, but do need a scientific review. Although a multitude of *in vitro* assays are used and are informative during drug discovery, not all are sufficient for regulatory use. For a number of reg-

ulatory questions that need to be addressed during drug development, a number of non-animal alternatives are currently being used as part of an integrated assessment. More *in vitro*/alternative assays will be accepted and used if they can answer the regulatory questions with equal or greater relevance than currently used assessments.

W 2575 Learnings from Early-Safety Assessment in Pharmaceutical Industry: Pfizer

Y. Will. *Pfizer, Groton, CT.*

Late stage attrition is costly and should be avoided at all times. Many companies are now positioning safety assessment much earlier in drug development process when chemical matter is abundant. Technology utilized ranges from *in silico* models to *in vitro* assays, often in a high throughput format. We and others have had a lot of learning's in the past few years and will continue to learn in the forth coming years. For example, we have learned that it is not enough to use a human hepatocytes to predict human toxicity. We learned that predictivity is hard to define, and depends on a well annotated set of compounds with clinical outcomes. We have also learned that toxicity is rather multifactorial, so one assay is often not enough to predict toxicity. Organ toxicity in general is difficult to predict. We have learned that projection of plasma drug concentration is needed to change intrinsic values to truly predictive margins. We have learned that it's easier to detect a hazard, than to predict true risk. It is important to take away that not all technologies should be applied to all projects. Technologies vary widely in their validation state, throughput and cost. A thoughtful combination of validated and emerging technologies is crucial in identifying the most promising candidates to move to proof-of-concept testing in humans.

W 2576 Chemicals and Agrochemicals Perspective on Moving Toward 21st Century Safety Assessment

M. Covaro. *Dow Agrosciences, Abingdon, United Kingdom.*

Global regulatory testing of new agrochemical active substances includes a large number of animal tests with redundancy of evaluated end-points. Alternative methods and use of the 3Rs principles would allow moving towards a 21st century paradigm for safety assessment of agrochemicals and industrial chemicals. Integrating computational approaches with *in vitro* and toxicokinetic end-points provides clear opportunities to: A) obtain more information from each study, using fewer animals; B) remove studies that have low/no impact on hazard identification; C) perform exposure-based and relevant data for identification of Mode of Action, human relevance and risk assessment purposes; D) remove acute toxicity testing for formulations, where appropriate. Differences between agrochemicals and industrial chemicals' safety assessment will be presented.

W 2577 Next Challenge for Cosmetic Industry in Europe: Systemic Toxicity Case Study

G. Ouedraogo. *L'Oreal, Paris, France.* Sponsor: K. Tsaioun.

Cosmetics in Europe are subject to the animal testing ban according to EU Cosmetic regulation (CE 1223/2009). Addressing repeated-dose systemic toxicity with non-animal methods is a substantial research challenge: a state of the art review published in 2011 by Adler et al., concluded that a full replacement of the animal tests used for repeated dose/reproductive and developmental toxicity testing was not available and the timeframe for a full replacement could not be clearly estimated. Since then, several research initiatives have made progress in Europe and overseas in developing different approaches covering bioactivity with *in vitro* and *in silico* methods, adverse outcome pathways and exposure prediction. This project builds on the data available in ToxRefDB and generated in ToxCast to model repeated-dose systemic toxicity in a pragmatic fashion. Other features related to the chemical structure like chemotypes and molecular descriptors were also considered. Each building block of the model will be highlighted and the overall performances obtained will be presented. Applications of the model will also be discussed.

W 2578 A Paradigm Shift to Advance the Relevance of Toxicology for Regulatory Policy

D. Mandrioli. *Cesare Maltoni Cancer Research Center Ramazzini Institute, Bentivoglio (Bologna), Italy.* Sponsor: K. Tsaioun.

Advancing the science of toxicology for regulatory policy involves not only investment in improving scientific methods, but also a change in how toxicological science delivers relevant evidence to policymakers. The difficulties in translating the evidence provided by science into policy are documented. In 2014 the US NRC addressed this impasse directly, issuing recommendations for the use of Systematic Reviews (SR) pioneered by the Cochrane Collaboration (CC), for assessing evidence from toxicology for regulatory policy. This will address some of the most obvious problems in toxicology: an aversion to transparency and "cherry picking" of studies for review. As shown in the work of CC, decision-making has been advanced by SRs that adhere to transparency and clearly defined and validated methods for assembling ALL of the relevant literature, extracting and evaluating data with a priori criteria for identifying sources of systematic error (bias). Most importantly, this is not current practice in toxicology. Some of the criteria considered fundamental by toxicologists are not relevant to testing study quality and reliability. For example, GLP relates to keeping adequate records, not for ensuring appropriately designed or valid studies. The Klimisch Score over-values compliance with GLP and established test guidelines without addressing the important criteria of statistical rigor and attention to sources of bias as well as validity and relevance of the study design. In addition, more research is needed on the potential for conflicts of interest to introduce bias in toxicology studies as has been demonstrated by SR of clinical trials and observational epidemiology of tobacco. In conclusion, we argue that adherence to the philosophy and methods of CC can expedite adopting NRC recommendations. We can adopt or adapt some well tested methods from existing CC handbooks and focus our efforts on developing and validating new methods specific to toxicology, including strategies for identifying and accessing relevant toxicology information; integrating the diversity of study designs; considering both internal and external validity; and providing evidence based conclusions usable in both hazard identification and risk assessment. SR, properly conducted, hold promise for advancing both the science and practice of toxicology and providing a gateway for new science.

W 2579 A Paradigm Shift to Advance the Relevance of Toxicology for Regulatory Policy

E. Silbergeld. *Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Co-Presenter with Dr. Mandrioli

W 2580 Screening Chemicals for Neurotoxicity Outcomes—Using Large Datasets and Multiple Endpoints to Develop "Toxicity Profiles"

T. J. Shafer[1] and M. Behl[2]. [1]*US EPA, Research Triangle Park, NC and* [2]*NTP/NIEHS, Research Triangle Park, NC.*

The potential for neurotoxicity in adults and children following exposure to environmental chemicals remains a high public priority because of concerns that recent increases in the prevalence of neurological disorders (e.g., Parkinson's, ADHD, autism) may in part be due to chemical effects. In addition, neurotoxicity is one of the leading reasons for the failure of new drug candidates. Thus, the need for reliable and efficient screening tools to identify, prioritize, and evaluate chemicals for their potential to induce acute neurotoxicity in adults or developmental neurotoxicity (DNT) is well recognized. The past decade has thus seen increased efforts to develop high-throughput, high-content assays that are useful to screen compounds for neurotoxicity or DNT. To date, assay development has focused on relatively small sets (5–20) of compounds. Recently, however several studies have evaluated the comparative neurotoxicity/DNT of larger (30–100) sets of compounds, in some cases using a battery of assays or endpoints. This provides the opportunity to begin examining how such datasets can be used to develop toxicity profiles that better inform decisions regarding the potential neurotoxicity/DNT of chemicals. This workshop will present data from several studies examining larger numbers of compounds, discuss the strengths and limitations of the assays used to generate the data, and provide novel strategies to score relative biological activity across different assays. Attendees will gain a national and international perspective from academia, government, and industry using well-characterized high-throughput, high-content cell-based assays, and alternate animal models, spanning across multiple aspects of development, neurodevelopment, and neural activity to evaluate, compare, and contrast the biological activity in large sets of chemicals. The use of this information to

build "toxicity profiles" can help inform decision-making related to neurotoxicity and DNT. The introduction will lay out the agenda of the workshop, including the overall goals, speaker lineup, and the intended outcome. The workshop will commence with an introduction by Tim Shafer, who will introduce the concept of using data from multiple chemicals and endpoints to inform decisions about chemical neurotoxicity, and briefly provide an overview of each presentation. A major liability in drug development and evaluation of chemical toxicity is the development of seizures. Our first speaker will discuss how multiple endpoints extracted from high-content recordings of neural network activity using microelectrode arrays can be used to identify and classify seizurogenic compounds early in the drug development process. The second speaker will discuss how combination of neural network recordings, and plasma and brain metabolome data can be used for early identification of several classes of neurotoxic compounds. The next speaker has used a combination of transcriptomics and toxicity in peripheral sensory (dorsal root ganglion) nerve cells to determine patterns of toxicity responses that can identify different classes of chemicals. The last two speakers will focus on methods to identify and prioritize environmental chemicals with potential for DNT/NT. The fourth speaker will present the approach that the US EPA has used to evaluate a battery of assays to collectively predict DNT and NT associated with unknown compounds. One of the major challenges in comparing results across different assay platforms is the choice of a common metric to assess chemical effect since different assays use disparate approaches to define potency. Using an 80-compound library that was tested for different DNT and NT endpoints by several researchers, the last speaker will present an approach that provides a uniform and robust metric for comparison of the biological activities across different assays to rank compounds by their toxicity for further hazard characterization *in vivo*. The workshop is designed to provide an overview of the current status and challenges in the DNT field, including the current national and international regulatory guidelines, and novel strategies that are currently being implemented in the field.

W 2581 A Novel High-Throughput *In Vitro* and Multivariate Spike Train Analysis Platform for Drug Neurotoxicity and Method of Action Identification

C. Strock. *Cyprotex, Watertown, MA.*

Seizurogenic neurotoxicity is a significant liability in drug development and evaluation of chemical toxicity. Currently available *in vitro* assays cannot predict this toxicity effectively or with any significant throughput. Therefore, the development of a high-throughput *in vitro* assay to screen compounds for seizurogenic potential would be of great value. Here we demonstrate the use of a 48-well microelectrode array (MEA) to screen for seizurogenic compounds using rat cortical neurons. Spike train activity was recorded and the results were computed using custom data analysis algorithms/Matlab scripts. The development of a panel of spike train descriptor statistics for activity (firing rate), synchronization, and inter-spike and inter-burst organization has been proven to effectively differentiate between activity patterns of negative and positive control compounds. This assay has been validated on over 50 compounds and has the ability to screen dozens of compounds per week in dose curve and many more as a single dose. Furthermore, we have demonstrated the sensitivity of this assay to distinguish the method of action (MOA) between several classes of pro-convulsant compounds, enabling us to classify compounds into subgroups within a broader range of neurotoxic liabilities. These methods are illustrated by considering several examples within three classes of seizurogenic compounds: GABAA antagonists (e.g. gabazine), glycine receptor antagonists (e.g. strychnine), and NMDA receptor agonists (e.g. NMDA). All showed significant changes in one or more descriptor endpoints that were characteristic of their MOA. These results illustrate the power within the high-throughput rat cortical neuron MEA data for predicting compound induced neural toxicity.

W 2582 Identification of Neurotoxicity Using *In Vitro* and *In Vivo* Metabolomics Data

H. Kamp. *BASF, Ludwigshafen, Germany*. Sponsor: T.J. Shafer.

Early identification of neurotoxicity can reduce testing costs and have other benefits. We have developed a testing strategy for the identification of neurotoxicity based on *in vitro* "neurons on a chip" as well as *in vivo* metabolomics data. BASF has developed a database of *in vitro* electrophysiological measurements (Microelectrode Array, MEA) for more than 70 test substances of diverse chemical classes and neurotoxic mechanisms. The data demonstrate an accuracy of the method of 75%, which is similar to reports from other working groups. BASF has developed over the last years an *in vivo* plasma metabolomics data base including the metabolome data of more than 700 compounds including several neurotoxicants. Based on these data, we defined several specific patterns for different neurotoxic effects. Among these are central nervous system-related effects (such as modulation of the GABA- or dopamine-receptor) or effects related to the sympathetic nervous system. Moreover, metabolome data from the brain for more than ten neurotoxic insecticides have been generated. These data show that neurotoxic insecticides can change the metabolome in brain tissue specifically for the neurotoxic modes of action, whereas the plasma metabolome is not affected. The case studies will illustrate that the combination of these different data/ methodologies allow the early identification of several classes of neurotoxic compounds.

W 2583 Incorporation of Transcriptome Data in Functional Screening Assays of Peripheral Nervous System Toxicity for Improved Compound Grouping and Mechanistic Understanding

M. Leist. *University of Konstanz, Konstanz, Germany*.

In vitro approaches to identify toxicants acting on the peripheral nervous system or its development have been hampered by limited availability of respective human cell supply. Such cells can now be generated in large amounts by leveraging the potential of stem cell technology. Here, two examples are presented on the generation of human cells otherwise not accessible for toxicity testing, and the use of such cells to establish screening assays for developmental toxicants. First, neural crest cells have been generated from human pluripotent stem cells, and a neural crest migration assay has been established to test functional disturbances of these cells. Findings from the screening of two libraries will be presented: (1) a library created by the National Toxicology Program (NTP), which comprised 80 compounds including known neurotoxicants and environmental compounds with unknown neurotoxic potential was used; (2) the ESNATS 30 compound library of mainly drug-like compounds was also screened. After successful screening, transcriptomics data of the hits have been used for functional grouping of environmental toxicants. A second assay was established to measure peripheral sensory neuron toxicity (PeriTox test). For this purpose, human dorsal root ganglia-like cells were generated from pluripotent cells. Then, a test system for evaluation of specific neurotoxicity was established, evaluated, and used for compound screening. The about 30 hits were then also tested in a central nervous system toxicity test. This comparison showed that the two assays had distinct toxicant detection capacities and that the PeriTox test was more sensitive for peripheral neurite toxicants.

W 2584 Evaluating the Ability of *In Vitro* Assays Based on Key Events in Neurodevelopment to Predict Developmental Neurotoxicity (DNT)

W. Mundy. *US EPA, Research Triangle Park, NC.*

Because of the multitude of potential molecular targets for chemical disruption in the developing nervous system, our laboratory has developed *in vitro* assays that measure chemical disruption of key neurodevelopmental processes. These include proliferation, differentiation, apoptosis, axonal and dendritic development, synaptogenesis and network formation. The performance of each assay was validated using "training set chemicals" known to alter the individual neurodevelopmental process. To evaluate the ability of these assays, individually and collectively, to predict DNT, we have tested a set of over 50 chemical "DNT reference chemicals" and negative controls. For each assay, concentration-response relationships are established for both the neurodevelopmental endpoint of interest (e.g. proliferation) as well as cell health. This allows for the determination of the selectivity of each chemical on neurodevelopmental processes. Chemical effects were diverse in that some hit only a single endpoint, while others altered multiple endpoints. No single assay was predictive of DNT, but based on a selectivity score the battery of assays was able to discriminate between negative controls and DNT reference compounds. (This presentation does not reflect Agency Policy).

W 2585 Using HTS Data for Decision-Making for Further *In Vivo* Testing

M. Behl. *NTP/NIEHS, Research Triangle Park, NC.*

One of the major challenges in comparing results across different assay platforms is the choice of a common metric to assess chemical effect. We evaluated a battery of well-characterized high throughput, high content cell-based assays, and alternate animal models, covering multiple aspects of development, neurodevelopment, and neural activity using

a 80-compound library to screen for potential developmental and adult neurotoxicants. The assays include (but are not limited to) neural crest migration, neuronal proliferation and neurite outgrowth using human derived induced pluripotent stem cells from controls and patients with Parksinsons-disease, neuronal firing, and whole organiasms such as zebrafish. Previously, each of the assays used disparate approaches to define potency, including threshold (e.g. when the response exceeds 30% of the control) values, lowest effective concentration (LEC), benchmark dose (BMD) modeling with a predefined level of biological response (i.e., 1 standard deviation (SD) of vehicle control-group responses), etc. Because these divergent assays measure different endpoints, comparing data across the various platforms is a challenge. To address this concern, instead of using a predefined response level, we adopted a BMD-like approach with an assay-specific response level. The approach intends to identify the lowest response level (i.e. noise level) where variance in the point-of-departure (POD) estimation is substantially reduced. This approach provides a uniform and robust metric for comparison of the biological activities across the various assays, and is important in ranking compounds by activity for further *in vivo* testing.

2586 Tox21 Challenge To Build Predictive Models of Nuclear Receptor and Stress Response Pathways As Mediated by Exposure to Environmental Toxicants and Drugs

M. Xia[1] and R. Huang[2]. [1]*NCATS/NIH, Bethesda, MD and* [2]*NCATS/NIH, Bethesda, MD.*

The Tox21 program, a collaboration between the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP), the US Environmental Protection Agency's (US EPA) National Center for Computational Toxicology (NCCT), the National Institutes of Health (NIH) National Center for Advancing Translational Sciences (NCATS), and the US Food and Drug Administration (US FDA), has generated quantitative high-throughput screening (qHTS) data (>50 million data points) on a library of 10K compounds, including environmental chemicals and drugs, against a panel of nuclear receptor and stress response pathway assays during its production phase (phase II). NCATS organized a Challenge that asked a "crowd" of researchers to use these data as the training set for this modeling challenge to elucidate the extent to which the interference of biochemical and cellular pathways by compounds can be inferred from chemical structure data. This Challenge represents a groundbreaking new direction for toxicity testing and is intended to help improve the understanding of how chemicals could disrupt biological pathways and result in toxicity. Specifically, the computational models generated from this Challenge can be applied to predict the potential of those environmental chemicals with limited information to disrupt cellular nuclear receptor and stress response pathways. The computational models built within this Challenge are expected to improve the community's ability to prioritize novel chemicals with respect to potential concern to human health. The first presentation will give an overview of the Tox21 program, including the selection and validation of qHTS assays. The second presentation will summarize the Challenge in terms of participation (378 submissions from 18 different countries worldwide), data sets, the scoring process, and the performance of the computational models. The third to sixth presentations are from the Challenge winners, who will discuss the specific methods they employed to develop the winning models.

2587 Safety and Efficacy of a Novel Fenugreek Seed (*Trigonella foenum-graecum*, Fenfuro™) Extract in Patients with Type-2 Diabetes

D. Bagchi. *Research & Development, Cepham Inc, Concord, CA.*
Sponsor: D. Bagchi.

Trigonella foenum-graecum seeds are known to exhibit potent antioxidant, hypoglycemic and nephroprotective activities, as well as serve as excellent membrane stabilizer especially because of their content of novel furostanolic saponins. Our previous studies exhibited the broad spectrum safety and efficacy of Fenfuro, a novel *Trigonella foenum-graecum* seed extract enriched in furostanolic saponins, in type 2 diabetes (T2D) in rats. This multi-center, randomized, placebo-controlled, double-blind, add-on clinical study evaluated over a period of 3 months the efficacy of Fenfuro (500 mg bid) in 154 male and female subjects (25-60 years) with T2D. This study examined the body weight, blood pressure and pulse rate, as well as the efficacy of Fenfuro on fasting and post-prandial plasma sugar, HbA1c and fasting and post-prandial C-peptide levels. Fenfuro caused significant reduction in both fasting plasma and post-prandial blood sugar levels. Approximately 83% of the subjects reported decrease in fasting plasma sugar levels in the Fenfuro group as compared to 62% in the placebo group, while 89% of the subjects demonstrated reduction in post-prandial plasma sugar levels in the Fenfuro-treated group as compared to 72% in the placebo group. HbA1c levels were reduced in both placebo and treatment groups. The decrease in HbA1c levels was significant in both groups as compared to respective baseline values. A significant increase in fasting and post-prandial C-peptide levels compared to the respective baseline values, were observed, while no significant changes in fasting and post-prandial C-peptide levels were observed between the two groups. Extensive blood chemistry analyses exhibited the broad spectrum safety of Fenfuro. Furthermore, 48.8% subjects reported reduced dosage of anti-diabetic therapy in Fenfuro-treated group, while 18.05% reported reduced dosage of anti-diabetic therapy in the placebo-group. In summary, Fenfuro proved safe and efficacious in ameliorating the symptoms of T2D in humans.

2588 Exposure to Imidacloprid Promotes Obesity and Type 2 Diabetes in C57BL/6J Mice

Q. Sun[3], X. Xiao[3], Y. Kim[3], D. Kim[3], K. S. Yoon[1], J. M. Clark[3] and Y. Park[3]. [1]*Department of Biological Sciences and Environmental Sciences Program, Southern Illinois University, Edwardsville, IL;* [2]*Department of Mathematics and Statistics, Umass Amherst, Amherst, MA and* [3]*Department of Veterinary and Animal Sciences, Umass Amherst, Amherst, MA;* [4]*Food Science, Umass Amherst, Amherst, MA.*

Imidacloprid, a neonicotinoid insecticide, has been reported to promote adipogenesis and cause insulin resistance *in vitro*. Thus, the purpose of the current study was to determine the effects of imidacloprid on development of obesity and type 2 diabetes using a mouse model. Moreover, interaction between dietary fat content and imidacloprid was determined. Imidacloprid (0.06, 0.6, or 6 mg/kg body weight/day) was mixed in low-fat (4% w/w) or high-fat (20% w/w) diet, and given to male C57BL/6J mice ad libitum for 12 weeks. Mice fed with high-fat diet containing all three doses of imidacloprid gained significantly more body weight and adipose tissue weight, but no effects of imidacloprid on these were observed in low-fat fed animals. Imidacloprid treatment with high-fat diet resulted in impaired insulin tolerance, glucose tolerance and increased serum insulin level compared to mice fed with high-fat control diet. Non-significant increase of insulin resistant markers were observed with imidacloprid treatment over control in low-fat diet. However, imidacloprid treatment resulted in higher Homeostatic model assessment (HOMA-IR) score in both high-fat and low-fat fed animals. In addition, imidacloprid with high-fat diet resulted in increased levels of triglyceride, glucose, leptin, insulin, and cholesterol, but not with low-fat diet. These results suggest significant interactions between dietary fat content and imidacloprid on development of obesity and type 2 diabetes *in vivo*. This project is supported by NIH R21ES023128.

2589 Effects of Food Grade Carrageenan on Inflammatory Signaling Pathways in a Human Hepatoma Cell Line (HepG2)

H. M. Baas and J. M. McKim. *IONTOX, Kalamazoo, MI.*

Carrageenan (CGN) is a common food additive used primarily as a thickener and stabilizing agent. Animal safety studies using high molecular weight CGN have demonstrated that CGN is safe for human consumption. Recently, it has been reported that CGN can bind to Toll-Like Receptor 4 (TLR4) and induce proinflammatory pathways in HepG2 cells. These results have not been repeated by other laboratories, which is important in order to understand potential human risk. Poligeenan (PGN) (formerly referred to as degraded CGN) with Mw 10-20 kDa has been associated with intestinal inflammation in animals, but is not used as a food additive. CGN is a natural polysaccharide (Mw of 200-800 kDa), is resistant to breakdown in the gut, and is not absorbed across intestinal epithelium. In addition, CGN and PGN bind tightly to proteins, which would reduce cellular exposure *in vitro* and *in vivo*. Therefore, the aim of this study was to carefully evaluate whether CGN can cross intestinal cells *in vitro* and induce an inflammatory response in HepG2 cells. Intestinal absorption was measured using a standard Caco-2 permeability model. HepG2 cells were seeded into 96-well plates at a density of 15,000 cells/100 µL/well in the presence and absence of 10% fetal bovine serum (FBS). The cells were exposed to lambda-CGN (0.1, 1.0, and 10 µg/mL) for 24 hr. Positive control groups included PGN (Mw = 19 kDa, 10 µg/mL) and lipopolysaccharide (LPS) (10 ng/mL and 100 ng/mL). Three (media/ exposure) conditions were tested; 1) compound plus media with 10% FBS for 24 hr, 2) compound plus media with 10% FBS for 20 hr followed by compound plus FBS free media for 4 hr, and 3) compound plus serum free media for 24 hr. Following the exposure times the cells were either evaluated for ATP or used to prepare total RNA. Cellular ATP was used to monitor cell viability while changes in the expression of interleukin-8 (IL-8), as determined by qRT-PCR, was used to assess TLR-4 signaling. Following the exposure period, LPS produced a concentration

dependent increase in IL-8 expression and this was greatest in media 3 (>10-fold). PGN induced IL-8 by 8-fold in both media 2 and 3, but had no effect on IL-8 when FBS was present (media 1). Lambda-CGN had no effect on IL-8 induction in any of the media formulations. Cell viability was greater than or equal to 80% of untreated controls. CGN did not cross the Caco-2 cell barrier and did not cause cytotoxicity. In conclusion, CGN is not absorbed from the gut and does not induce a proinflammatory response mediated by TLR-4 binding in HepG2 cells.

PL 2590 Influence of Serum Lycopene on Lung Cancer Mortality: An 18-Year Follow-Up Study of a National Cohort

E. Afriyie-Gyawu, S. Akuse, O. Adeyinka and J. Zhang. *Biostatistics, Georgia Southern University, Statesboro, GA; Environmental Health Sciences, Georgia Southern University, Statesboro, GA; Epidemiology, Georgia Southern University, Statesboro, GA.* Sponsor: E. Afriyie-Gyawu.

A potent antioxidant, lycopene, is the most abundant and naturally-occurring carotenoid in tomatoes and tomato-based foods. It is frequently found in pink grapefruit, watermelon, guava, and papaya; and it provides fruits and vegetables their red and pink colors. Lycopene promotes high levels of free-radical scavenging compared to other carotenoids such as β-carotenoids. It inhibits cellular proliferation, angiogenesis, and metastasis through multiple biochemical pathways. Epidemiological studies suggest that consumption of lycopene-rich foods is associated with decreased risk of prostate, lung, breast and GI tract cancers. To our knowledge, influence of lycopene on lung cancer mortality has not been characterized. Thus, the objective of this study was to determine whether there is an association between serum lycopene levels and lung cancer mortality. A retrospective cohort study was conducted with 14,358 adult participants in phase II of the National Health and Nutrition Examination Survey III (1991-1994) (NHANES III). This served as baseline and was correlated with the National Death Index database for a 15 year (1991-2006) follow-up study. Hazard ratios (HRs) for all-cause and cancer-related deaths for individuals with high, medium, and low serum lycopene levels were calculated using the Cox Proportional Hazards Regression Model. The unadjusted HR of deaths associated with low serum levels (25% cutoff) of lycopene were 1.67 (95%CI=1.24-2.23) and 1.00 (ref). After adjusting for multiple risk factors such as age and sex, the HR for lung cancer mortality were 1.00 (ref) and 1.45 (95%CI=1.08-1.96) for low serum levels (25% cutoff). Adjusted HR for lung cancer death using 3-level categorization (and adjusted for fruits and vegetables) was 1.67 (95%CI=1.03-2.71) for low vs. high levels of lycopene. Also, adjusted HR for lung cancer death using 3-level categorization (and unadjusted for fruits and vegetables) was 1.68 (95%CI=1.04-2.72) for low vs. high levels of lycopene. Results suggest that high serum lycopene levels significantly reduce the risk of death from lung cancer. Thus, not only does lycopene decrease risk of lung cancer development, it also decreases lung cancer mortality. Further studies are needed to explain the physiological mechanisms of this phenomenon.

PL 2591 Metabolism, Mutagenicity, and DNA Adduct Formation of the Naturally Occurring Alkenylbenzene beta-Asarone

A. T. Cartus, S. Stegmüller, K. Berg and D. Schrenk. *Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany.*

beta-Asarone (bA, (Z)-1,2,4-trimethoxy-5-(1-propen-1-yl)benzene; CAS No 5273-86-9) is a propenylic alkenylbenzene found in several plants, e.g. Acorus calamus. bA-containing plant materials are used to flavor foods, and are active ingredients in traditional plant medicines. Thus, human exposure results primarily from the intake of bitters and teas, as well as from Calamus-containing medicines and plant food supplements. Although many (positive) pharmacological properties/effects of asarone isomers are described in the literature, bA was found to be carcinogenic in rodents (liver, duodenum) when given daily or in a single dosage. Early experiments indicated that bA is not activated via hydroxylation and sulfonation as it is the case for known hepatocarcinogenic allylic alkenylbenzenes like estragole or methyleugenol. Because the mode of mechanism of the activation of bA in not known, we investigated the metabolism of bA in liver microsomes and human cytochrome P450 (CYP) enzymes, the mutagenicity of bA and its metabolites in the Ames fluctuation assay and the DNA adduct formation in primary rat hepatocytes. We found that side-chain epoxidation (leading to diols and a ketone) was by far the most dominating metabolic route of bA in liver microsomes and human CYP enzymes. bA was mutagenic in the Ames test (+S9 mix), as well as the synthesized bA-epoxide (-S9 mix). Furthermore, we were able to synthesize and characterize a bA epoxide-derived DNA adduct with deoxyguanosine. This DNA adduct was detected and quantified in a dose-dependent manner in the DNA of rat hepatocytes incubated with bA. Our results strongly indicate that bA is genotoxic with the side-chain epoxide being its ultimate carcinogen.

PL 2592 *Ginkgo biloba* Leaf Extract Induces DNA Damage by Inhibiting Topoisomerase II Activity in Human Hepatic Cells

N. Mei[2], Z. Zhang[2], S. Chen[2], J. Xuan[2], X. Guo[2], V. N. Dobrovolsky[2] and L. Guo[2]. [1]*Division of Biochemical Toxicology, US FDA/NCTR, Jefferson, AR and* [2]*Division of Genetic and Molecular Toxicology, US FDA/NCTR, Jefferson, AR.*

Ginkgo biloba leaf extract has been shown to increase the incidence in liver tumors in mice in a 2-year bioassay conducted by the National Toxicology Program. In this study, the DNA damaging effects of *Ginkgo biloba* leaf extract and many of its constituents were evaluated in human hepatic HepG2 cells and the underlying mechanism was determined. A molecular docking study revealed that quercetin, a flavonoid constituent of *Ginkgo biloba*, showed a higher potential to interact with topoisomerase II (Topo II) than did the other *Ginkgo biloba* constituents; this *in silico* predication was confirmed by using a biochemical assay to study Topo II enzyme inhibition. Moreover, as measured by the Comet assay and the induction of γ-H2A.X, quercetin, followed by keampferol and isorhamnetion, appeared to be the most potent DNA damage inducer in HepG2 cells. In Topo II knockdown cells, DNA damage triggered by *Ginkgo biloba* leaf extract or quercetin was dramatically decreased, indicating that DNA damage is directly associated with Topo II. DNA damage was also observed when cells were treated with commercially available *Ginkgo biloba* extract product. Our findings suggest that *Ginkgo biloba* leaf extract- and quercetin-induced *in vitro* genotoxicity may be the result of Topo II inhibition.

PL 2593 Chemopreventive Effects of Anthocyanin-Rich Fruit *Chrysobalanus icaco* L. Against *In Vivo* Doxorubicin-Induced Genotoxicity and *In Vitro* TNF-α-Induced Inflammation

V. P. Venancio[1,3], M. R. Almeida[1], A. Z. Mercadante[2], C. M. Marzocchi-Machado[1], M. L. P. Bianchi[1], S. Talcott[3], S. U. Mertens-Talcott[3] and L. M. Antunes[1]. [1]*Department of Clinical Analyses, Toxicology and Food Science, University of São Paulo, Ribeirao Preto, Brazil;* [2]*Department of Food Science, State University of Campinas, Campinas, Brazil and* [3]*Department of Nutrition and Food Science, Texas A&M University, College Station, TX.*

Chrysobalanus icaco L. (CI) is an underutilized/underinvestigated anthocyanin-rich fruit found in tropical areas around the globe. Due to the presence of anthocyanins in this fruit, it is possible that CI may exert pharmacological activities. This study aimed at investigating CI fruit *in vivo* antigenotoxicity and antioxidant activity against doxorubicin (DXR)-induced damage, and its anthocyanins *in vitro* anti-inflammatory activity in inflamed normal colon and colon cancer cell lines (CCD-18Co and HT-29, respectively). CI showed antigenotoxicity by decreasing both Tail Moment and Tail Intensity in peripheral blood comet assay and reducing the frequency of micronuclei in peripheral blood and bone marrow cells at 100, 200 and 400 mg/kg/day after a 15-day experiment, when compared to DXR (15mg/kg i.p.) group. The fruit exerted *in vivo* antioxidant activity (100, 200 and 400 mg/kg/day) by decreasing the reactive oxygen species (ROS) generation in peripheral blood neutrophils. Anti-inflammatory activity of CI anthocyanins was demonstrated by the decreased TNF-α, IL-1β, IL-6 and NF-κB gene expressions at 20.0 mg/L GAE in HT-29 colon cancer cell lines and the downregulation of IL-1β, IL-6 and TNF-α proteins at 20 mg/L GAE in CCD-18Co normal colon cells previously inflamed with TNF-α. These chemopreventive effects may involve the major anthocyanin, carotenoid and phenolic compounds identified in CI fruit (petunidin-3-glucoside, all-trans-lutein and ellagic acid derivative). The results indicate CI fruit possess pharmacological activities, demonstrated by the decreased *in vivo* DNA damage and ROS generation and the *in vitro* lower expression of inflammatory biomarkers. Financial support: FAPESP, CNPq, CAPES.

2594 Role of Nrf2 on Expression of Genes for Detoxification and Xenobiotic Metabolism Enzymes (XMEs) in the Liver of Sodium Fluoride-treated Adult Female Zebrafish (*Danio rerio*)

D. Mukhopadhyay and A. Chattopadhyay. *Department of Zoology, Visva-Bharati University, Santiniketan, India.*

Sodium fluoride (NaF) is a natural pollutant in water. Due to anthropogenic activities its concentration is gradually increasing and causing threat to the aquatic organisms including fish. Therefore it is important to study the effect of NaF and its toxicity in the liver of adult female zebrafish at cellular and molecular level. In the present study, zebrafish were exposed to 7.5, 15 and 30 mg NaF/L for one month. Treatment with 15 mg NaF/L was continued for three months also. Cellular viability and induction of apoptosis were also checked in 15 mg NaF/L treated fish. Histopathology revealed ultrastructural alterations in treated liver tissue evidenced by hyperplasia, cytoplasmic degeneration and heteropycnosis. Toxicological parameters like GSH, MDA content, CAT, SOD and GST levels were altered. The mRNA level of Cyp1A1, Nrf2, Nqo1, Ho1, MnSod and Gpx were upregulated whereas Keap1 was down regulated in the treated groups in comparison to control. The transcriptional expression of Cu/ZnSod, Gst, Hsp70 and Cat genes were also altered in a time dependent manner both at 30 and 90 days exposure periods. The protein levels of Nrf2 and Keap 1 confirmed their important role in regulating fluoride induced oxidative stress. Trypan blue exclusion assay, DNA fragmentation assay, Hoechst and EtBr/AO staining as well as flow cytometry (FACS) data showed that the dead cells comprised largely of necrosis. In our study, it was observed that there was no marked elevation of Ucp2 mRNA in liver. This could be correlated with the low induction of apoptosis in hepatocytes. In conclusion, fluoride can modulate the expression of biomarker genes at low concentration in aquatic organism, particularly in fish. Keywords: zebrafish liver; sodium fluoride; oxidative stress; xenobiotic metabolizing enzymes; Nrf2

2595 An Imaging-Based RNAi Screen Identifies Novel Regulators of Nrf2 Activation

S. Hiemstra, M. Niemeijer, B. Herpers, S. Wink and B. van de Water. *Toxicology, Leiden University, Leiden, Netherlands.*

The Nrf2 anti-oxidant response pathway is a key mechanism in protecting cells against reactive oxygen species-mediated toxicity. To quantitatively assess the Nrf2 pathway activation we combine live cell imaging with GFP-tagging of individual pathway components (sensor: KEAP1; transcription factor: Nrf2; downstream targets: SRXN1, NQO1 and HMOX1). Using high throughput confocal imaging we have measured the dynamics of Nrf2 activation and established the concentration time course of Nrf2 pathway activation with different activators: CDDO-me, diethylmaleate and tBHQ. In order to provide full understanding of the upstream regulators of the Nrf2 pathway, we applied a siRNA-based knock-down screen using the Srxn1-GFP reporter as a downstream target of Nrf2. We screened all individual kinases, phosphatases, ubiquitinases and transcription factors for their involvement in Nrf2 activation, a total of ~3300 individual genes. After knock-down, CDDO-me was used to activate Nrf2 followed by quantification of Srxn1-GFP expression using automated high throughput imaging and image analysis. Candidate Nrf2 pathway regulators that upon KD enhanced (58 genes) or inhibit (19 genes) the CDDO-me-induced Srxn1 upregulation were validated with single siRNAs. Candidate hits included ATF3, BRD4 and RXRA which have previously been associated with Nrf2 regulation. All candidate hits were further validated with additional compounds which are known to activate the Nrf2 pathway: diethylmaleate, acetaminophen and diclofenac. Furthermore, most of the candidate genes were confirmed using two other independent BAC-GFP reporters for Nrf2 activation: NQO1-GFP and HMOX1-GFP. In addition, intracellular glutathione levels were affected following knock-down. Interestingly, the novel hits show distinct patterns in SRXN1 live cell dynamics and some of the novel hits also directly affect Nrf2 stability. Moreover, candidate hits were linked to susceptibility to xenobiotic induced cytotoxicity. Our future direction will focus on more in depth mechanistic studies to unravel the function of our newly discovered individual KEAP1/Nrf2 pathway regulators. This work is supported by the IMI MIP-DILI project (grant agreement n° 115336)

2596 The Development of a Mechanistic Systems Biology Model for Nrf2 and Oxidative Stress to Understand Homeostatic Control and Adaptive Responses to Low Doses of Oxidative Compounds

S. Cooper[5], M. E. Andersen[2], P. L. Carmichael[5], K. Castle[5], R. A. Clewell[2], C. Courage[5], S. Das[1], S. Glavin[5], G. Jain[4], B.-w. Huang[2], P. Jones[5], A. Middleton[5], N. M K[1], N. R[1], J. Shao[5] , K. Subramanian[1], B. van der Water[3], J. Vethamanickam[4], S. Windebank[5] and A. White[5]. *[1]Strand Life Sciences, Bangalore, India; [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC; [3]Leiden University, Leiden Academic Centre for Drug Research, Leiden, Netherlands; [4]Safety & Environmental Assurance Centre, Unilever, Bangalore, India and [5]Safety & Environmental Assurance Centre, Unilever, Bedfordshire, United Kingdom.*

Determining the tipping-point when homeostatic regulatory mechanisms become saturated and shift from an adaptive to an adverse state is critical to define regions of safety for chemical exposure. Oxidative stress is used as a case study to develop these approaches to enable the progression of risk assessment through mechanistic understanding of human relevant systems. A range of high content *in vitro* assays including redox status, Nrf2 translocation, GSH levels & macromolecule damage were used to understand the dynamic alterations in molecular responses to oxidative stress. Time- and dose-response data was generated in both hepatocyte & keratinocyte cell lines utilising 4 oxidative stress-inducing compounds (H2O2, tBHQ, Curcumin & Quercetin) with different mechanisms of action. The data highlights a temporal alteration in Nrf2 signaling response, moving from a low dose transient perturbation to a longer lasting activation state at higher doses. Similar dose-dependent transient responses for other biomarker responses are also observed, suggesting that a biological pathway altering dose (BPAD) can be defined based on the dose-dependent dynamics of cellular repair and recovery. As part of this case study a dynamic systems biology model covering the core components of the Nrf2 signalling network with GSH quenching has been developed using activation and deactivation in Nrf2 signalling as an indicator of the capacity of the cell to recover (adapt) from an oxidative stress insult. In order to enable the translation of the *in vitro* data to *in vivo* relevance, distributions in the levels of relevant biomarkers (such as GSH & MDA) under both normal physiological & adverse conditions have been taken from the literature and analyzed.

2597 Alternative Flame Retardants and Related Compounds Activates Oxidative Stress Nrf2 Pathway and Aryl Hydrocarbon Receptor *In Vitro*

A. K. Rosenmai, A. Oskarsson and J. Lundqvist. *Biomedical Science and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

The toxicity and persistence of polybrominated diphenyl ether (PBDEs) flame retardants (FRs) have led to legislative actions and the phase-out of a number of PBDEs. Consequently, alternative flame retardants are used. However, little is known about the toxicity of some of these compounds, which pose challenges with respect to assessing human risk. We have studied the potential toxicity of alternative FR and related compounds, using the nuclear factor erythroid 2-related factor 2 (Nrf2) and aryl hydrocarbon receptor (AhR) reporter gene assays using HepG2 cells. Oxidative stress is involved in a wide range of toxicological endpoints. Nrf2 is a regulator in the cellular defense against oxidants and a key event in the oxidative stress pathway is the release of Nrf2. AhR is a ligand-activated transcription factor regulating xenobiotic-metabolizing enzymes, which in some cases lead to formation of genotoxic substances. We found that 4-bromophenol (4-BP) and tetrachlorobisphenol A (TCBPA) caused increased Nrf2 and AhR activity at non-cytotoxic concentrations. 4-BP and TCBPA induced the Nrf2 activity with 200–300 % compared to the vehicle control, with a lowest-observable effect concentration (LOEC) of 50 µM. The effects on AhR activity was more potent with LOECs of 6.3 and 12.5 µM for 4-BP and TCBPA, respectively. Additionally, the changes in AhR activity with exposure were in the range of 300–400 %. In conclusion, our data show that some alternative flame retardants and related compounds can activate an oxidative stress pathway and the aryl hydrocarbon receptor. These novel findings add important information to the discussion about the toxicity and safety of these compounds.

2598 The Possible Mechanism of Chemopreventive Action of 4-Methylthio-3-Butenyl Isothiocyanate in Human Esophageal Epithelial Cells

T. Hirata[3,4], Y.-M. Cho[3], I. Suzuki[1,3], T. Toyoda[3], J.-i. Akagi[3], Y. Nakamura[2], S. Numazawa[4] and K. Ogawa[3]. *[1]Pathogenetic Veterinary Science, United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan; [2]Department of Food Sciences and Nutritional Health, Kyoto Prefectural University, Kyoto, Japan; [3]Department of Pathology, National Institute of Health Science, Tokyo, Japan and [4]Department of Pharmacology, School of Pharmacy, Showa University, Tokyo, Japan.*

4-Methylthio-3-butenyl isothiocyanate (MTBITC) which is included in white radish is expected to have inhibitory effects on carcinogenesis. However, there are few reports proving the mechanism of it. In the present study, we investigated the effect of MTBITC on human esophageal epithelial cells, Het-1A cells and KYSE 510 cell line. In Het-1A cells, we investigated whether MTBITC (0–10 μM) induces cytoprotective genes, including phase II enzymes which may detoxify mutagens and prevent formation of DNA adducts. qPCR analysis revealed that 10 μM MTBITC increased mRNA expressions of HMOX1, NQO1 and GCLC. And protein level of Nrf2, the transcription factor regulating these genes, was increased in Het-1A cells exposed to MTBITC. In addition, we found that MTBITC generated reactive oxygen species (ROS)-mediated Nrf2 activation in Het-1A cells. On the other hand, in KYSE 510 cells, we investigated whether MTBITC induces cell death in order to eliminate cancer cells. MTBITC (20–40 μM) induced cell death and decreased Bcl2 protein expression and induced cleaved-caspase 3, suggesting that the cell death occurred through mitochondrial apoptotic pathway in KYSE 510 cells. While, Chk1 phosphorylation and decreased p27 protein expression, which are regulators of cell cycle, were also observed. These results indicate that MTBITC has potential for preventing esophageal carcinogenesis, by modifying carcinogen metabolism via induction of phase II enzymes through ROS production and elimination of cancer cells via induction of apoptosis and cell cycle arrest.

2599 All-*Trans*-Retinoic Acid Mediated Stress Protein Induction and Modification Afford Cytoprotection Against Renal Injury

J. M. Sapiro, K. L. Burrell and S. S. Lau. *SWEHSC, Dept Pharmacol/Toxicol, College of Pharmacy, University of Arizona, Tucson, AZ.*

Chemical-induced nephrotoxicity is a major cause of acute kidney injury. Pretreatment of LLC-PK1 cells with all-trans-retinoic acid (ATRA, 25 μM, 24 hr) affords cytoprotection against p-aminophenol (PAP), and 2-(glutathion-S-yl)hydroquinone-induced necrosis. In contrast, co-treatment of LLC-PK1 cells with these toxicants and ATRA does not provide cytoprotection, suggesting that critical cell survival signaling pathways need to be activated prior to toxicant exposure to provide cytoprotection. Interestingly, ATRA did not alter PAP-induced ROS generation and had a modest effect on Nrf2 protein expression suggesting other signaling proteins beyond ROS reduction contribute to ATRA protection. Additional protective mechanisms against cellular stress were therefore investigated. During ATRA-mediated pretreatment, the ER stress proteins Grp78 and p-eIF2α were induced (2-fold) in a time-dependent manner (24 and 4 hr respectively). In addition to influencing organelle stress proteins in the ER, ATRA rapidly (15 min) induced levels of the cellular stress kinases p-ERK and p-AKT with maximum levels achieved at 30 min. Although p-ERK levels declined to basal levels over the next 4 hr, elevations in p- AKT were maintained for 24 hr, correlating with the time required for maximal ATRA protection. Moreover, induction of these stress kinases was observed at concentrations of ATRA (10 and 25 μM) required for cytoprotection. Interestingly, inhibition of p-ERK with PD98059 reduced the ability of ATRA to provide protection against PAP toxicity, implying a possible role for p-ERK or downstream target genes in the protective effects of ATRA. We subsequently demonstrated that ATRA caused a rapid (1-4 hr) 3-fold increase in cyclin D1 levels. Collectively, stress proteins in the UPR (Grp78, p-eIF2α), stress kinases (p-ERK, p-AKT), and mitogenic/proliferative effects (cyclin D1) appear to work cooperatively to afford ATRA protection against renal cell injury by ameliorating protein damage and cellular stress (ES006694, T32ES007091,ES016578).

2600 Metallothionein as One of Nrf2 Downstream Genes Plays an Important Role in Sulforaphane Prevention of Diabetes-Induced Cardiac Remodeling and Pathogenic Damage

Y. Cheng[1,3], L. Kong[1,3], H. Wu[2,3], Z. Xu[1] and L. Cai[3]. *[1]The First Hospital of Jilin University, Changchun, China; [2]The Second Hospital of Jilin University, Changchun, China and [3]Pediatrics, University of Louisville, Louisville, KY*. Sponsor: L. Cai.

We have shown that sulforaphane (SFN) protected from diabetic cardiomyopathy (DCM) in both T1DM and T2DM mouse models along with induction of both Nrf2 and metallothionein (MT). Here we defined whether SFN protects from DCM via either Nrf2 or MT and even both. Both Nrf2-knockout (KO), MT-KO, and their wild-types (WT, C57BL/6J or 129S1) mice were used to induce T2DM by feeding a high-fat diet (HFD) for 3 months followed by a small dose of streptozotocin (STZ). Age-matched control mice were fed a normal diet (ND) for the same period. Both T2DM and control mice were treated with or without SFN at 0.5 mg/kg five days a week for 4 months along with continual feeding with either HFD or ND diet, respectively. SFN prevented diabetes-increased cardiac oxidative damage, inflammation, fibrosis, and hypertrophy along with increases in Nrf2 and MT expressions in the WT mice. Both diabetic Nrf2-KO and MT-KO mice exhibited greater cardiac damage than diabetic WT mice, and SFN did not protect in Nrf2-KO mice, and only partially protected in MT-KO mice, from diabetes-induced cardiac damage. SFN cannot induce MT expression in Nrf2-KO mice, but can stimulate Nrf2 function in MT-KO mice. The present study demonstrates for the first time that Nrf2 plays the pivotal role in SFN cardiac protection from T2DM. SFN-induced MT expression is Nrf2-dependent and also is a key player in Nrf2-mediated cardiac protection induced by SFN from T2DM.

2601 Preventive Effect of Broccoli Sprout Extracts on the Development of DCM in Db/db T2dm Mice Is Associated with the Up-Regulation of Nrf2 Expression and Function

Z. Xu[1], J. Sun[2] and L. Cai[1]. *[1]Kosair Children's Hospital Research Institute, Louisville, KY and [2]The Cardiovascular Center, The First Hospital of Jilin University, Changchun, China.*

The aim of this study was to investigate whether consumption of broccoli sprouts extracts (BSE) can be used as natural sulforaphane (SFN, a Nrf2 activator) to up-regulate systemic NFE2-related factor 2 (Nrf2) for the prevention of diabetic cardiomyopathy (DCM). Five-months-old db/db and age-matched wild type (WT) mice were fed BSE (containing 2 mg SFN per tablet, from Source Naturals Company) or SFN by gavage every other day for 3 months. Both db/db and WT mice were divided into four groups: vehicle (0.1 ml/10g), BSE-low dose (0.5 mg/kg), BSE-high dose (1.0 mg/kg), and SFN (0.5 mg/kg) as a positive control. After 3 months treatment, cardiac function was assessed by echocardiography. Cardiac hypertrophy, fibrosis, inflammation, and oxidative injury were assessed by Western blot, real-time PCR, and histopathological examination. Both BSE and SFN significantly prevented diabetes-induced cardiac dysfunction, hypertrophy, and fibrosis (increased accumulation of collagen and expression of connective tissue growth factor). BSE and SFN also prevented diabetes-induced cardiac oxidative damage and inflammation. Like SFN, the positive control of Nrf2 activator, BSE significantly up-regulated Nrf2 expression and transcription activity, reflected by increased Nrf2 nuclear accumulation and the mRNA and protein expression of Nrf2 downstream genes. These results indicated that the prevention of DCM by BSE at high dose is similar to those of SFN group, suggesting that BSE can be used as SFN nature product to up-regulate Nrf2 expression and function for the prevention of DCM. Since BSE is a health supplement, the potential for its clinical application for diabetic patients is promising.

2602 Sulforaphane Protects Against SO2 Inhalation-Induced Lung Injury by Activation of the Nrf2 Pathway

M. Rojo de la Vega Guinea[3], G. Luo[3], B. Chau[3], S. Tao[3], C. Lantz[3], T. Wang[3], S. Black[3], E. Chapman[3], J. G. Garcia[3] and D. D. Zhang[3]. *[1]Arizona Respiratory Center, University of Arizona, Tucson, AZ; [2]Pharmacology and Toxicology, University of Arizona, Tucson, AZ and [3]Translational and Regenerative Medicine, University of Arizona, Tucson, AZ.*

Sulfur dioxide (SO2) is a toxic gas used in the sulfuric acid and wood pulp industries. SO2 has a strong pungent odor, and when it comes in contact with water in mucous membranes of the airways it forms sulfurous acid (H2SO3) causing irritation. Several industrial and transportation accidents have been document where people were exposed to very high

concentrations of SO2 and developed acute lung injury (ALI). Currently, the therapies for SO2-induced lung injury are limited to palliative measures such as anti-inflammatory steroids or mechanical ventilation (MV) for those with respiratory failure. Activation of the antioxidant and anti-inflammatory Nrf2 pathway with chemopreventive compounds has been proven to effectively protect against several air pollutants and ALI. In the present study, the efficacy of Nrf2 activation by the chemopreventive compound sulforaphane (SF) was tested in an acute SO2 inhalation mouse model. Mice were pretreated with 12.5 mg/kg SF or vehicle control (i.p.) for 24h. Mice were exposed to 1000 ppm SO2 for 1 h to simulate a catastrophic high dose exposure, then were allowed to recover for 12 h before obtaining bronchoalveolar lavage (BAL), blood plasma, and lung tissues. Untreated mice had significantly greater inflammation than SF-treated mice, as indicated by higher BAL total protein concentration, total cell count, and neutrophil infiltration. Histological examination of lung tissues showed that SF attenuated pathological alterations of the lung in response to SO2 exposure. Microarray analyses showed that SO2 deregulated 817 genes, most of which correspond to immune cell signaling. Together, these results indicate that SF reduces SO2-induced lung inflammation. *In vitro* studies in human pulmonary artery endothelial cells (HPAECs) treated with H2SO3 indicated that this compound produced reactive oxygen species (ROS) and compensatory upregulation of Nrf2. SF treatment caused greater Nrf2 upregulation and decreased ROS. All these results demonstrate that Nrf2 pathway activation by SF is an effective strategy to decrease SO2-induced lung injury by attenuation of inflammation and ROS production.

PS 2603 An Improving *In Silico* Phototoxicity Prediction: It Showed High Concordance Equivalent to the Results of Inter-Laboratory Validation Study for 3T3 NRU-PT

Y. Haranosono, S. Nemoto, M. Kurata and H. Sakaki. *Senju Pharmaceutical, Kobe, Hyogo, Japan.*

Previously, we reported an *in silico* prediction method for *in vitro* 3T3 NRU-PT phototoxicity. Our method has been established with π electron number and HOMO-LUMO Gap which were related to photo-absorption and photochemical reaction. In this presentation, we investigated to add new descriptors of phototoxicity for improvement the concordance. We calculated potential energies of chemicals as new descriptor candidates which were considered to be contributed on chemical reactivity with/without photo irradiation. As the result of investigation, "stretch-bend" and "dipole/dipole" were found as the new descriptors of phototoxicity. What the key-point of our *in silico* method was defining the "gray zone" between "positive zone" and "negative zone", exclusive. To define above zones, we set two cut-off lines for each descriptor. In this study, we sampled compounds randomly to set training set for minimize the effect of dataset dispersion. It was achieved that a new *in silico* method using HOMO-LUMO Gap, π electron, and new descriptors showed high concordance (84.4%) with high specificity (83.0%) for 96 compounds. This result indicated that the potential energy of chemical was one of factors for *in vitro* phototoxicity likewise photo-absorption and photo-reaction. Finally, this study strongly indicated that the concordance of new *in silico* was equivalent to the results of inter-laboratory validation study for 3T3 NRU-PT (87.5%). The ICH S10 guideline was established for photo-safety assessment in drug development at 2013. In the guideline, it is listed on photo-absorption, distribution to light-exposed tissue, and photo-reactivity as the primary assessment, and 3T3 NRU-PT assay as the *in vitro* assay. Although 3T3 NRU-PT assay has high sensitivity, it is known to give many false positive (low specificity). Because our method can predict 3T3 NRU-PT with high concordance, we suggest that the *in silico* is conducted as "the first assessment" before the primary assessments and *in vitro* 3T3 NRU-PT. For example, when the *in silico* shows positive for a compound, we judge that the compound has a potential of phototoxicity and some *in vivo* tests are recommended (the *in vitro* is skipped). When the *in silico* shows negative, we recommend the primary assessments on S10. Because new *in silico* needs only chemical structure, it is suitable for the first assessment of phototoxicity.

PS 2604 Computational Models to Estimate *In Vivo* Activity Concentrations From Tox21 HTS Data

N. S. Sipes[2], J. F. Wambaugh[1], R. Pearce[1], B. A. Wetmore[3], M. J. DeVito[2] and S. S. Ferguson[2]. *[1]NCCT/US EPA, Research Triangle Park, NC; [2]NTP/NIEHS, Research Triangle Park, NC and [3]The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Tox21 and ToxCast efforts provide *in vitro* high-throughput screening (HTS) AC50 values for thousands of chemicals. Moving these data to predict whether chemical-biological interactions observed *in vitro* are likely to occur *in vivo* is challenging. We hypothesize using a modified approach applied by the FDA for drug interaction studies, Cmax/AC50 (maximal *in vivo* blood concentrations over *in vitro* mechanistic Tox21 assay potencies), will provide useful approximations of blood concentrations required to produce *in vivo* interactions. 7381 Cmax values were estimated for Tox21 chemicals using physiologically-based toxicokinetics models for HTS data (HTTK R-package), with both *in silico* (ADMET-Predictor) and *in vitro* (measured) parameters of intrinsic clearance and fraction unbound to human plasma. 339 chemicals with measured human Cmax values correlated with estimated Cmax values, R2=0.6. A preliminary focus on 16 chemicals with PPARγ agonist activity, human exposure and Cmax data showed that the Cmax/AC50 estimates using *in vitro* or *in silico* parameters were similar and accurately identified three glitazones as likely for *in vivo* interaction at therapeutic doses. As these doses will not be common for environmental compounds, real-life environmental exposure predictions were obtained from a rapid heuristic method using information from urine analytes, which does not explicitly differentiate pharmaceutical exposure (Wambaugh JF et al 2014). Cmax levels where Tox21 assay responses are predicted to interact *in vivo* were calculated across 3706 chemicals and X Tox21 assay endpoints. The Cmax values were generally found to far-exceed environmental exposures (one-time), but repeat cumulative exposures and certain occupational situations could increase exposure levels to these ranges. Model uncertainties and out of domain compounds also exist and need to be characterized. This approach has shown promise toward estimating *in vivo* interaction concentrations for HTS data. This abstract does not reflect official NTP or EPA views.

PS 2605 The Novartis Translational Study Data Warehouse— A 21st Century View for Our Animal Data

P. Marc[4], F. Hahne[4], D. Selinger[2], M. Wilbert[2], J. Boisclair[2], R. Kikkawa[6], E. Skuba[6], R. Stull[6], K. Wuersch[4], T. Brunner[6], J. Demchak[6], R. Galicia[6], B. Huang[2], K. Therrien[2], J. Walker[2] , T. Welker[4] and P. Bouchard[2]. *[1]NIBR informatics, Novartis, Basel, Switzerland; [2]NIBR informatics, Novartis, Cambridge, MA; [3]NIBR informatics, Novartis, East-Hanover, NJ; [4]PreClinical Safety, Novartis, Basel, Switzerland; [5]PreClinical Safety, Novartis, Cambridge, MA and [6]PreClinical Safety, Novartis, East-Hanover, NJ.*

With CDISC-SEND coming of age, we recently released a new generation of data warehouse, bringing preclinical safety data to the fingertips of our researchers in real time. Novartis has a long history of preclinical data warehouses, with our first global data warehouse released more than a decade ago in 2003. Building on that previous work, we created one of the largest animal study repositories in the world. Currently the database contains data from more than 7,000 animal studies, 200 clinical studies, and data from other safety relevant datasets (*in vitro* data, pharmacovigilance data, etc.). User adoption is critical to the success of such a system. To maximize ease-of-use, our user interface was developed in close collaboration with our PreClinical Safety colleagues, with special attention given to the query interface, data visualization, and integration with other applications. We present here the user interface features and main graphical interactive plots.

PS 2606 Translating Computational Toxicology Data Through Stakeholder Outreach and Engagement

M. Linnenbrink, R. Thomas and K. Crofton. *US EPA, Research Triangle Park, NC.*

US EPA has been using *in vitro* testing methods in an effort to accelerate the pace of chemical evaluations and address the significant lack of health and environmental data on the thousands of chemicals found in commonly used products. Recently, EPA released high-throughput screening data on thousands of chemicals. These chemicals were screened for potential health effects in over 700 high-throughput screening assay endpoints. As part of EPA's commitment to transparency, all data is accessible through the Chemical Safety for Sustainability Dashboard (iCSS). Policy makers and stakeholders can analyze and use this data to help inform decisions they make about chemicals. Use of these new datasets in risk decisions will require changing a regulatory paradigm that has been used for decades. EPA recognized early that a communications and outreach strategy was needed to parallel the research and aid with the development and use of these new data sources. The goal is to use communications and outreach to increase awareness, interest and usage of analyzing and using these new chemical evaluation methods. To accomplish this, EPA employs numerous communication and outreach activities including the Communities of Practice webinars, materials describing this research (webpages, fact sheets, videos), proactive scientific media outreach, educational events such as workshops, establishing hundreds of research collaborations worldwide, and actively requesting feedback from stakeholders. Using stakeholder feedback, EPA has designed a data analysis pipeline, devel-

oped a series of tutorial videos and improved the iCSS dashboard. This presentation will provide an overview of EPA's communications and outreach strategy including: communication and outreach goals; summaries of research collaborations with outside organizations; outreach and communication strategies implemented (workshops, webinars, websites, data challenges, etc); discussion about which strategies were the most and least successful; and plans for future communication and outreach efforts. This abstract does not necessarily reflect US EPA policy

2607 Identification of Absorption, Distribution, Metabolism, and Excretion (ADME) Genes Relevant to Steatosis Using a Gene Expression Approach

S. C. Suarez[1,2] and H. El-Masri[2]. [1]*Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN and* [2]*US EPA, Research Triangle Park, NC.*

Absorption, distribution, metabolism, and excretion (ADME) mediate chemical concentration and activation of molecular initiating events of Adverse Outcome Pathways (AOPs) in cellular, tissue, and organ level targets. In order to better describe ADME parameters and how they modulate potential hazard posed by chemical exposure, our goal is to create a database of ADME related genes and functional information. Given the scope of this task, we began using hepatic steatosis as a case study. To identify ADME genes related to steatosis, we used the publicly available toxicogenomics database, DrugMatrix. This database contains standardized rodent chemical exposure data for over 2000 exposure conditions, along with differential gene expression data and corresponding associated pathological changes. We examined the entire chemical exposure microarray data set, including arrays gathered from the 25 chemical exposures resulting in pathologically confirmed (severity score>1) incidences of steatosis. From this differential gene expression data, we utilized differential and co-expression analyses to identify gene changes resulting from the chemical exposures leading to steatosis. We then utilized pathway enrichment analysis to further expand our gene set and identify differentially expressed genes (DEGs) related to ADME. These DEGs include genes involved in the metabolism and clearance of chemical exposures (CYP Family, FMO, GST family, and UGT family genes), lipid metabolism and transport (APO family, CD36, LDLR, PPAR Family, and FASN genes), and energy regulation. These results demonstrate the utility of a disease-focused case study as one method to populate our ADME gene database, thus increasing our understanding of the connection between exposure and AOPs. This abstract does not necessarily reflect US EPA policy.

2608 Predicting Dermal Penetration for Toxcast Chemicals Using *In Silico* Estimates for Diffusion in Combination with Physiologically Based Pharmacokinetic (PBPK) Modeling

M. V. Evans[3], M. E. Sawyer[1], K. Isaacs[3] and J. Wambaugh[3]. [1]*Department of Mathematics, University of Southern New Hampshire, Manchester, NH;* [2]*ORD/NCCT, US EPA, Research Triangle Park, NC and* [3]*ORD/NERL/HEASD/EMR, US EPA, Research Triangle Park, NC;* [4]*Pharmacokinetics, US EPA, Research Triangle Park, NC.*

With the development of efficient high-throughput (HT) *in vitro* techniques, a strategy has been outlined to correlate *in vivo* toxicity with HT measurements. ToxCast is an EPA research program for screening environmental chemicals with HT hazard tests. Under the analogous ExpoCast program, predictive models are being developed to estimate exposures and target doses to allow, in combination with ToxCast hazard predictions, risk-based chemical prioritization. Dermal exposure may be a major route of exposure for many ToxCast chemicals, such as those found in consumer products. Mathematical diffusion models allow for *in silico* calculation of dermal diffusion constants using empirical equations that include physico-chemical characteristics. Lag time (Tlag) is a constant defining the length of time needed for dermal penetration to start. Tlag is related to the diffusion constant and molecular weight. Estimating diffusion and lag time constants with established mathematical models, we calculated Tlag values for about 2,000 chemicals listed as part of Toxcast Phase I and II. Values in Tlag ranged from 0.1 hours to 2663 hours in length. The shorter Tlag values indicate that this chemical can be rapidly absorbed, therefore shorter Tlag chemicals could be prioritized for study due to their ability to cross the skin barrier rapidly. The application of Tlag as a prioritization tool will depend on activity patterns and other lifestyle data. Ultimately, this *in silico* approach can be used for characterization of dermal absorption processes in forward exposure-dose PBPK and reverse dosimetry models in ExpoCast and elsewhere. Future research will compare diffusion constants and dermal parameters generated *in silico* with *in vivo* or *in vitro* dermal kinetic datasets. (The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA.)

2609 Predicting Rat Chronic Systemic Toxicity Using *In Vitro* Bioactivity Data

J. Liu and I. Shah. *NCCT/US EPA, Durham, NC.*

Predicting the adverse effects of chemicals across different target organs is a challenging problem. We used high-throughput screening (HTS) data from ToxCast and rat chronic guideline test data from ToxRefDB on 369 environmental chemicals to develop predictive models of systemic toxicity. Chemicals were defined positive for a target organ toxicity if lowest effect levels (LELs) were observed, and negative otherwise. The ten most frequent target organs for adverse effects included: liver (240/369), kidneys (188/369), adrenals (104/369), brain (64/369), heart (68/369), lungs (79/369), ovaries (55/369), spleen (97/369), testes (86/369), and thyroid (90/369). Predictive models for each target organ toxicity were built using six machine learning algorithms: linear discriminant analysis (LDA), Naïve Bayes (NB), support vector machines (SVM), classification and regression trees (CART), k-nearest neighbors (KNN) and the ensemble of these classifiers (ENSMB). Predictive performance was evaluated using 10-fold cross-validation testing and in-loop, filter-based, feature subset selection. The best balanced accuracies for predicting target organ toxicities were: liver/0.80±0.08, kidneys/0.78±0.08, lungs/0.78±0.10, ovaries/0.77±0.11, heart/0.76±0.10, spleen/0.76±0.09, testes/0.76±0.09, thyroid/0.76±0.09, brain/0.75±0.10, and adrenals/0.75±0.08. SVM, CART and ENSMB classifiers performed the best, and nuclear receptor activation was frequently found in highly predictive classifiers. Our findings demonstrate how machine learning techniques can be used to predict rodent systemic toxicity from ToxCast and ToxRefDB data. This abstract does not represent US EPA policy.

2610 *In Vitro* to *In Vivo* Extrapolation (IVIVE) for Drug Induced Liver Injury (DILI)—A Genome-Wide Analysis

Z. Liu and W. Tong. *Division of Bioinformatics and Biostatistics, National Center of Toxicological Research (NCTR) US FDA, Jefferson, AR.*

Many drugs causing human toxicity are not predicted by the preclinical studies, which led efforts to explore *in vitro* systems to supplement or even replace animal uses such as REACH in Europe and Tox21 and ToxCast in U.S. It was demonstrated that gene expression data from *in vitro* toxicogenomics (TGx) systems can be predictive for chemical-induced toxicity. One aspect of *in vitro* to *in vivo* extrapolation (IVIVE) is to examine how gene expression patterns from an *in vitro* system resemble to these from *in vivo* systems. We assessed IVIVE for DILI with TGx using a classic *in vivo* assay and two *in vitro* methods. The *in vivo* data were obtained from the rat liver treated with 131 chemicals for 28 days while two *in vitro* data were generated using either rat or human primary hepatocytes treated with the same chemicals for 24 hours. We developed a Drug-Pair Ranking (DPRank) method for the IVIVE analysis. First, for a given TGx system, pairwise similarity between any two drugs was calculated based on their overlapped DEGs. Next, the drug-drug pairs were ranked, from the most similar to least similar. Lastly, the IVIVE potential was determined based on the order preservation of similarity rankings between an *in vitro* method and the *in vivo* platform using a receiver operating characteristic (ROC) analysis. A better IVIVE concordance was obtained for rat primary hepatocytes (AUC=0.71) than for human primary hepatocytes (AUC=0.58), indicating species difference playing a critical role in IVIVE. When limiting the analysis to only these drugs causing severe DILI, the IVIVE concordances were improved for both rats (from 0.71 to 0.76) and humans (from 0.58 to 0.62). The similar analyses were also conducted for drug classes (e.g., therapeutic uses), DILI patterns (i.e., hepatocellular, cholestatic, and mixed), and others. The results indicated that, for study of DILI, rat or human *in vitro* TGx methods hold the promise to replace the expensive rat *in vivo* study. Moreover, the proposed DPRank methodology can be applied to other datasets to assess IVIVE such as Tox21 and ToxCast.

2611 Abstract Withdrawn by Author.

2612 Linear Mixed Models Approach to Identify Gender Specific Transcriptional Responses to Polychlorinated Biphenyls (PCBs)

A. Espin Perez[1], T. M. de Kok[1], S. A. Kyrtopoulos[2] and J. C. Kleinjans[1]. *[1]Maastricht University, Maastricht, Netherlands and [2]National Hellenic Research Foundation, Institute of Biology, Medicinal Chemistry and Biotechnology, Division of Organic and Medicinal Chemistry, Athens, Greece.* Sponsor: H. van Loveren.

Environmental exposures are linked to changes in the level of gene expression. Polychlorinated biphenyls (PCBs) are hormonally active organochlorines and classified as xenoestrogens. They have been described as carcinogens due to its ability to interfere in processes controlled by hormones and produce modifications in growth factors, promoting cancer in diverse organs like breast, kidney, reproductive system, pancreas lung and brain. EnviroGenomarkers is a European FP7 research project* consisting on 649 subjects from the Northern Sweden Health and Disease Study and the European Prospective Investigation into Cancer and Nutrition cohorts (www.envirogenomarkers.net). In this study, we run mixed models from a population of 277 non-smoker females and 186 non-smoker males in order to find associations with gene expression and exposure to PCBs, correcting for confounders and random effects. After adjusting for differences in gene expression across cohorts, the analysis has been split into two according to sex due to differences observed with regard to the response to exposure. 9220 single genes have been found associated with PCBs in total in the subpopulation of non-smoker females after correcting for multiple testing (FDR 0.05). We identified mainly cell cycle, transcriptomics and translation-related pathways deregulated, in particular oxidative stress, immune response and neurological diseases-related pathways. No association between transcriptome levels and exposure to PCBs in males has been found. Some PCB exposure levels are significantly different among the two populations. However, the strong response in females is consistent after adjusting for it by removing those low exposed females that generated such differences. The large number of genes in the female subpopulation suggests different biological response mechanisms to PCBs, which may be related that the average age of females was 53.31 ± 7.81 years, an age at which changes in the hormonal regulation occur e.g. possible menopausal processes. Ongoing cross-omics analysis of transcriptomics and methylation are in process in order to bring light to the gender-specific responses. * Research support by the European Union (Grants number 226756 and 308610)

2613 Percellome Project for Mechanistic Analysis of Chronic Toxicity by a New Concept of Repeated Dose Study

J. Kanno, S. Kitajima and K.-i. Aisaki. *Division of Cellular & Molecular Toxicology, National Institute of Health Sciences, Tokyo, Japan.*

The Percellome Toxicogenomics Project aims at reinforcing and eventually replacing the "safety factor" currently used for the extrapolation of experimental animal data to humans. Our project attempts to comprehensively identify the transcriptomic networks induced by xenobiotics. In order to pile up certain amount of transcriptomic data, "Percellome" method was developed (BMC Genomics 7:64, 2006) to generate absolute copy numbers of each mRNA per one cell (in average). By this method, data from the Affymetrix MOE430 2.0 GeneChip are absolutized and visualized in 3-D graphs (time x dose x copy number per cell). Up to now, datasets of single dose studies of mouse liver (4 dose levels, 4 time points (2, 4, 8 and 24 hours after oral gavage), triplicate, 48 GeneChip data per chemical) on more than 140 chemicals are compiled. Here, we report the effect of pretreatment with repeated dose (up to 14 days) of carbon tetrachloride (CCl4) on a single dose study of a chemical. This study design is a derivative of a single dose study on a gene knockout mouse. Corresponding to the knockout status, wild type mice pretreated with repeated dose of a chemical is considered to be in a "chemically-induced transgenic state". Various chemicals include CCl4 itself, clofibrate and phenobarbital was challenged after pretreatment. Repeated dose of CCl4 induced suppression of baseline expression levels of genes related to ER stress and attenuated the effect of challenged chemicals. Analysis strategy by the new concept of repeated dose study, i.e. "chemically-induced transgenic state" broadens the view of chronic toxicity with a way to directly compare with the chemical dosing studies using gene modified mice. (The Percellome Projects are supported by the Health and Labour Sciences Research Grant, MHLW, Japan)

2614 Transcriptomic-Based Prediction of Sex-Related Susceptibilities to Drug-Induced Adverse Effects in F344 Rats

J. C. Fuscoe, K. Wang, Q. Shi and V. Vijay. *US FDA, National Center for Toxicological Research, Division of Systems Biology, Jefferson, AR.*

A crucial step in developing a safe and effective drug is assessing how the body processes the drug. During non-clinical drug development, a drug candidate is often evaluated in adult animals of a single sex (males). If there are age- and/or sex-differences in the enzymes that metabolize the drug, there may be unrecognized age- and/or sex-related differences in the disposition, safety, and efficacy of the drug. Drug metabolizing enzymes including transporters (DME/T) play a major role in a drug's detoxification, excretion, and/or activation, and thus differences in the DME/T expression profiles may play a key role in drug safety. Therefore, a rat (F344) model was used to identify differences in the basal hepatic transcriptional profiles of DME/T genes in adult males and females. A comprehensive list of 336 rat DME/T genes was prepared using Pharmapendium as a key resource. Rat liver gene expression data was obtained for 298 out of 336 DME/T genes. Genes were considered to be significantly differentially expressed between females (F) and males (M) if the t-test p-value <0.05 and fold ratio (F/M or M/F) >2. The expression of 29 genes was significantly differentially expressed between the sexes with 12 being more highly expressed in females and 17 being more highly expressed in males. The genes with highest expression in females compared to males were Abcc3, Cyp3a9, Sult2a1, Adh6 and Cyp2c12 with a range of fold-differences of 3 to 378. Genes with the highest expression in males compared to females were Cyp2a2, Sult1e1, Cyp2c11, Cyp2c13 and Cyp3a2 with a range of fold-differences of 127 to 3876. The 29 enzymes encoded by these differentially expressed genes metabolize more than 600 drugs. Based on these findings, the disposition of these drugs may be different in the two sexes. Confirmation of these results by *in vivo* studies may allow the prediction of sex-related susceptibilities to adverse drug events from easily obtained transcriptomics data.

2615 Use of Automated Tools to Facilitate Rapid Chemical Hazard Assessment

P. J. Beattie[1], J. Orchard-Hays[1], J. P. Rinkevich[1], M. C. Ruhter[1], C. A. Rudisill[2] and J. L. Tunkel[2]. *[1]SciVera LLC, Charlottesville, VA and [2]SRC Inc, North Syracuse, NY.*

There is a growing need on the part of product manufacturers to rapidly assess large numbers of chemicals for human and environmental hazard. Many chemicals have not undergone toxicological testing and therefore lack experimental data to help to determine characteristics of human or environmental concern. Computer software can enable clustering of chemicals by similar structures, then evaluate the chemicals within each group for representative toxicity data, and apply a read-across process to fill data-gaps within the group. Our project examined the utility of chemical structure clustering software combined with aggregated available chemical hazard data for a subset of the chemicals of interest in facilitating rapid assessment of chemicals with limited human and environmental health data. Our team used the EPA Chemical Assessment Clustering Engine (ChemACE) tool to cluster chemicals into groups with similar structural characteristics. Our dataset consisted of approximately 2200 chemicals and chemical-specific SMILES notation to indicate molecular structure. 1100 of these chemicals fit into 223-257 clusters, depending on the ChemACE settings used. Our team then evaluated clusters for hazard characteristics using the SciVera Lens® automated chemical assessment platform. This combination of platforms allowed us to easily compare the chemicals with experimental hazard data and determine if they could be used to model the hazards of chemicals within the same cluster that do not currently have experimental data available. Our conclusions were: 1) In clusters where a chemical was high hazard for a critical endpoint (e.g., carcinogenicity, mutagenicity, developmental toxicity), the process highlighted the need for careful evaluation of the other chemicals in that cluster; 2) The results clearly indicated hazard characteristic trends across a cluster allowing for higher confidence in the use of analogous data, and 3) Having the ability to view the chemical structures in ChemACE for the compounds in the cluster was helpful for understanding subtle differences that could impact potential toxicity.

2616 A Gene Expression Biomarker Accurately Predicts Estrogen Receptor α Modulation in a Human Gene Expression Compendium

N. M. VanDuyn[2,3], B. N. Chorley[3], R. R. Tice[1], R. S. Judson[3] and J. C. Corton[3]. [1]*NIEHS, Research Triangle Park, NC;* [2]*ORISE, Research Triangle Park, NC and* [3]*US EPA, Research Triangle Park, NC.*

The EPA's vision for the Endocrine Disruptor Screening Program (EDSP) in the 21st century (EDSP21) includes utilization of high-throughput screening (HTS) assays coupled with computational modeling to prioritize chemicals with the goal of eventually replacing current Tier 1 screening tests. The ToxCast program currently includes 18 HTS *in vitro* assays that evaluate the ability of chemicals to modulate estrogen receptor α (ERα), an important endocrine target. We propose microarray-based gene expression profiling as a complementary approach to predict ERα modulation and have developed computational methods to identify ERα modulators in an existing database of whole-genome microarray data. The ERα biomarker consisted of 46 ERα-regulated genes with consistent expression patterns across 7 known ER agonists and 3 known ER antagonists. The biomarker was evaluated as a predictive tool using the fold-change rank-based Running Fisher algorithm by comparison to annotated gene expression data sets from experiments in MCF-7 cells. Using 141 comparisons from chemical- and hormone-treated cells, the biomarker gave a balanced accuracy for prediction of ERα activation or suppression of 94% or 93%, respectively. The biomarker was able to correctly classify 18 out of 21 (86%) OECD ER reference chemicals including "very weak" agonists and replicated predictions based on 18 *in vitro* ER-associated HTS assays. For 114 chemicals present in both the HTS data and the MCF-7 compendium, the balanced accuracies (BA) were 95% and 98% for activation or suppression, respectively. In a comparison to *in vivo* data for ER activity, the biomarker accurately predicted 48 out of 56 chemicals (BA = 86%) evaluated in rat and mouse uterotrophic assays. These results demonstrate that this gene expression-based ERα biomarker can accurately identify ERα modulators in MCF-7 cells and could be applied as a potential "Tier 0" screening model prior to ToxCast/Tox21 EDC high-throughput screens. This abstract does not represent US EPA policy.

2617 Toxicity Data Landscape of Food Additives and Ingredients Under US FDA Jurisdiction

P. Volarath[2], L. Holt[2], K. Arvidson[2] and C. Yang[1]. [1]*Altamira LLC, Columbus, OH and* [2]*US FDA, College Park, MD.*

OFAS's main responsibility is to ensure the safety of all food additives/ingredients used in the United States. Currently, safety decisions are supported using traditional *in vitro* and *in vivo* toxicity data. With new advances in chemical-toxicity databases and *in vitro* assays, OFAS is beginning to examine potential applications of information-rich datasets, such as ToxCast, in the food additive/ingredient review process. The first step in this analysis was to identify the chemical overlap of the ToxCast database and OFAS's food additive/ingredient list. A list of the food additives, GRAS ingredients, and FCSs under OFAS's jurisdiction was compiled from the following sources: the Everything Added to Food in the U.S. database, website of effective FCN and GRAS notices, FCS impurity list, and FEMA GRAS substances. For those compounds that were identified in both the food additive/ingredient and ToxCast chemical lists, the traditional *in vitro* and *in vivo* data were extracted from internal FDA databases, and non-traditional *in vitro* data were extracted from the ToxCast database. For compounds that were unique to the food additive/ingredient list, the areas of data enrichment/deficiency were identified for purposes of prioritizing data harvesting for the enrichment of the CERES knowledgebase. Finally, the chemical space of the OFAS food additives/ingredients and ToxCast chemicals were explored using Toxprint Chemotypes, and the overlaps/gaps between the data sets were identified. Collectively, the results from these analyses provided insights into the toxicity data landscape of OFAS food additives/ingredients.Abbrs: OFAS (Office of Food Additive Safety), FCN (Food Contact Notification), FCS (Food Contact Substance), FEMA (Flavor and Extract Manufactures Association), GRAS (Generally Recognized as Safe), CERES (Chemical Evaluation and Risk Estimation System) Disclaimer: This study does not reflect the policies of US FDA nor does it endorse products used in this analysis.

2618 MoA Central: A Search Engine for Mechanism of Action & Toxicity Studies

D. W. Selinger[2], V. Shivashankar[2], A. Ghodssi[2], M. Larbaoui[3], M. Steeves[2], M. Wilbert[2], I. Mendelev[2], S. Litster[2] and P. Marc[3]. [1]*Biologics, Novartis Institutes for BioMedical Research, Cambridge, MA;* [2]*NX (Research Informatics), Novartis Institutes for BioMedical Research, Cambridge, MA and* [3]*Preclinical Safety, Novartis Institutes for BioMedical Research, Basel, Switzerland;* [4]*Preclinical Safety, Novartis Institutes for BioMedical Research, Cambridge, MA.*

We have developed a compound target search engine, called MoA Central, capable of searching a large & diverse corpus of data (currently >200 million data points and growing) for related compounds and their known and/or putative targets. These compounds and targets are then linked to related pathways, phenotypes, toxicities, preclinical histopathology, and clinical adverse events. Based on an initial small molecule query (which can include known or suspected metabolites), we identify structurally and phenotypically related compounds, and their putative targets. Our corpus of data currently includes biochemical activities, biophysical interactions, chemical-genetic relations, *in silico* target predictions, as well as compound-compound similarities (both structural and via several kinds of biological "fingerprinting"). Compounds and targets returned in the search results are integrated with data from a large number of additional sources including: pathway databases, GWAS studies, mammalian phenotype databases, safety/toxicology databases, and most recently, our internal preclinical histopathology data. In contrast to many predictive approaches, MoA Central search results are easy to interpret and completely transparent - all supporting data is returned as part of the search results themselves. We are currently using MoA Central for safety assessment and mechanistic studies across our entire drug discovery and development pipeline.

2619 Mechanistic Expert Call Datasets Provide *In Silico* Prediction of Teratogenicity for a Wider Chemical Space

A. Fowkes, J. Plante, A. J. Myden, A. Cayley and M. B. Surfraz. *Lhasa Limited, Leeds, United Kingdom.* Sponsor: C. Barber.

The scarcity of teratogenicity data and the cost of *in vivo* reproductive toxicity studies are driving the use of a wider range of assays, where the relationship between data and teratogenicity can be established. Using an adverse outcome pathway framework, key events can be mapped and supported by data from *in vitro* assays and *in vivo* studies. This mechanistic approach provides a clear rationale between a specific molecular initiating event (MIE) or assay and a toxicity endpoint. This poster describes the creation of purposeful mechanistic expert call datasets, which were subsequently used to create *in silico* alerts for the prediction of teratogenicity. Data from the public domain for two MIEs - oestrogen receptor modulation (ERM) and 5alpha-reductase inhibition (5aRI) - were analysed and categorised using KNIME, with an expert call for activity assigned using expert-derived thresholds to classify a compound as active, weakly active, equivocal or inactive. Expert intervention was required for ERM data points for which the automated system failed to generate an expert call, allowing improved accuracy and confidence in the data. The mechanistic dataset for each MIE was mined to extract knowledge, enabling the implementation of nine structural alerts for ERM and 15 structural alerts for 5aRI into Derek Nexus. The new structural alerts increased the sensitivity of Derek Nexus prediction for teratogenicity from 28% to 73% and 25% to 72%, for the ERM and 5aRI mechanistic datasets respectively. Datasets obtained from other sources were then used as evaluation sets to validate the corresponding predictive models. MIE-based mechanistic datasets created this way support faster structural alert development in a transparent *in silico* expert system like Derek Nexus and support integration into a database. *In silico* predictions for teratogenicity, will help identify potential toxicity hazards during early stage drug development and facilitate high-throughput prioritisation for toxicity testing.

2620 A Computational Analysis of the Association of Inorganic Arsenic Exposure and Diabetes

J. Cota, S. Tan, S. Novin and D. E. Johnson. *Nutritional Sciences and Toxicology, UC Berkeley, Berkeley, CA.*

The association of inorganic arsenic (iAs) in relation to Type 2 Diabetes Mellitus (T2DM) has been studied extensively in populations in Mexico, China, Denmark, Bangladesh, USA, Argentina, Chile, and elsewhere, all concluding that long-term exposure to iAs contributes to the development of T2DM. Our studies utilized a computational approach to find

chemical-gene linkages and molecular pathways that may help provide more insight towards understanding the effects of iAs on diabetes. We utilized sources such as STITCH, KEGG, and The Comparative Toxicogenomics Database (CTD) to obtain genetic and disease information and created maps to show chemical-gene interactions that may ultimately link to diabetes. Our findings show that iAs can affect important molecular events and pathways that would ultimately contribute to the pathology of diabetes. Such events include the inhibition of PPARγ, a gene important in adipocyte production, and ROS production/oxidative stress, which may impair beta-cell function. PDK (pyruvate dehydrogenase kinase) enzymes that are important for glycolysis are also affected by iAs exposure. Consequently, iAs has the potential to cause a significant impact in the onset for diabetes due to its ability to impact critical pathways and genes that are important for glucose uptake, insulin secretion, and adipogenesis. To explore the higher incidence of T2DM in populations of Mexican descent, we studied population and geographic contamination data from San Bernardino, CA correlated with specific Single-Nucleotide Polymorphisms (SNPs), such as AS3MT, a gene involved in arsenic methylation reactions, and CAPN-10, which encodes a protein involved in the secretion and action of insulin. These polymorphisms are found in people of Mexican descent and may contribute to the higher incidence in these populations. Thus, exposure to iAs may be important to consider in addition to health and nutritional factors when addressing the issue of diabetes.

PS 2621 QSARs for the Fiftieth Anniversary of RD50

Y. Alarie. *Environmental and Occupational Health, University of Pittsburgh, Bradfordwoods, PA.*

In 1966, Alarie, Y. (Arch. Env. Health 13: 433-449) published a bioassay to evaluate the potency of airborne chemicals (vapors and aerosols) as sensory irritants. The potency was expressed as the exposure concentration resulting in a characteristic decrease in respiratory rate by 50% (RD50) in mice. The bioassay became widely used and QSARs were obtained (Alarie, Y. 1973 CRC Crit. Rev. Toxicol. 2: 299-363). A large database (244 chemicals) was then analyzed by M. Schaper in 1993 (Am. Ind. Hyg. Assoc. J. 54: 488-544). A correlation (R2=0.78) was obtained using 89 industrial chemicals for which both RD50s and Threshold Limit Values (TLVs) were available as well as more recently with LOAELs and RELs (Kuwabara, Y. et al. 2007. Environ. Health Perspec. 115:1609-1616) with this database. From this database, RD50s were also available for 83 reactive (r) and 59 nonreactive (nr) volatile organic chemicals (VOCs) of industrial importance. These chemicals were evaluated using sets of chemical descriptors. Excellent QSARs were obtained for nrVOCs (Alarie, Y. et al. 1998. Arch. Toxicol. 72:125-140, and Luan, F. et al. 2006. Chemosphere 63:1142-1153). For rVOCs, their potency was explained by separating them on the basis of specific reactivity mechanisms. Recently, Gupta, S. et al. (Ecotoxicology 2015, 24:873-886) used the same VOCs database. First they were able to differentiate the rVOCs from the nrVOCs and then predict the RD50s for both groups. This was arrived at on the basis of decision tree classification followed by regression analysis using selected molecular descriptors. Thus the use of QSARs capable of predicting RD50s, LOAELs or RELs values for regulatory purposes, for r or nrVOCs is within reach. Not so yet for solids or for liquids of very low vapor pressure, encountered in the aerosol state. The following will be presented and discussed and will be available at www.pitt.edu~RD50 and www.RD50.com for investigators to use to further improve predictive models using various approaches: 1) Update of the Schaper 1993 database adding published RD50s since then and updating TLV values to 2015; 2) Update of the database for aerosols for further QSAR analysis.

PS 2622 Computational Modeling of a Proposed Mechanism of Toxicity for β-Methylamino-L-alanine (BMAA)-Induced Amyotrophic Lateral Sclerosis

D. Li, S. Miguel, A. Sun, J. Tran, J. Voong and D. E. Johnson. *Nutritional Sciences and Toxicology, UC Berkeley, Berkeley, CA.*

Beta-Methylamino-L-alanine (BMAA) is a neurotoxin produced in nature by cyanobacteria and is reported to accumulate in seafood. Several studies have associated BMAA exposure with Amyotrophic Lateral Sclerosis/ Parkinson's Disease (ALS/PD); however, there is a lack of information regarding how BMAA metabolism can contribute to its ability to induce ALS/PD in patients. In this study we propose a new putative mechanism to highlight how the BMAA metabolite, 2, 3-diaminopropionic acid (DAP), can selectively target for neurons in the motor cortex, leading to the potentiation of neurodegeneration. Our computational methods, which included ChemMapper, ChemMine, and the Human Protein Atlas, show that DAP, as verified through ChemMapper, can possibly bind to the active site of cystathionine beta-synthase by mimicking endogenous L-serine, thus inhibiting the production of cysteine at the two sites where cystathionine beta-synthase is expressed: in the cerebral cortex and in the liver. Our model proposed from these results suggests a two-pronged mechanism of toxicity in motor neuron cells, with BMAA-carbamate causing release of calcium ions in the neuronal cytoplasm with a corresponding increase in ROS generation, and with DAP inhibiting the synthesis of glutathione, a key cellular defense against ROS. Degeneration of motor neuron cells is a key pathological indicator of the progression of ALS/PD. This model proposed may further explain how BMAA can be selective for motor neuron degeneration and may explain why monkeys treated with BMAA show symptoms of liver toxicity.

PS 2623 Chemical Security Analysis Center: Chemical Prioritization Process for Predictive Toxicology Testing

J. Moser[1,2] and R. Jablonski[1,2]. [1]*Battelle Memorial Institute, Columbus, OH and* [2]*Chemical Security Analysis Center, Department of Homeland Security, Aberdeen Proving Ground, MD.*

A number of organizations are developing and utilizing computational and/or *in vitro* methods to predict human toxicity. However, existing predictive toxicology programs do not address all US Department of Homeland Security (DHS) requirements. The Chemical Security Analysis Center (CSAC) has instigated a predictive toxicology program that leverages existing programs to develop acute toxicity prediction models that combine *in vitro* toxicity testing with computational toxicology, applied specifically to the DHS mission and DHS chemicals of interest. DHS chemicals of interest are classified into toxidromes; the ultimate goal of the program is to provide a capability to accurately estimate human toxicity of representative chemicals within selected toxidromes. For predictive toxicology testing, a process was developed to prioritize DHS chemicals according to toxidrome. Toxidrome prioritization will be based upon: 1) results of the 2012 CSAC Chemical Terrorism Risk Assessment (CTRA), which ranks the DHS chemicals of interest by risk to public health; 2) quantity and quality of available data for DHS chemicals and other chemicals that are either structurally or functionally related; and 3) availability of applicable predictive toxicology models. To this end, CSAC: 1) considered public health baseline risk and weighted average consequence per attack to rank each toxidrome represented in the CTRA; 2) conducted surveys to identify available sources of data and data gaps; and 3) completed a survey of predictive toxicology technologies as a first step towards model evaluation. Although the prioritization process is not complete, the CSAC has selected the cholinergics toxidrome (acetylcholinesterase inhibitors) to be the initial "test case" for predictive toxicology studies because of high representation in the CTRA, abundant data available, common mechanism of action among the cholinergics, and useful model structures/descriptors that have been identified for computational modeling of cholinergic chemical toxicity.

PS 2624 Mining a Human Transcriptome Database for Nrf2 Modulators

J. P. Rooney[1] and J. C. Corton[2]. [1]*ORISE, US EPA, Research Triangle Park, NC and* [2]*US EPA, Research Triangle Park, NC.*

Nuclear factor erythroid-2 related factor 2 (Nrf2) is a key transcription factor important in the protection against oxidative stress. We developed computational procedures to enable the identification of chemical, genetic and environmental modulators of Nrf2 in a large database of human microarray data (~38,000 transcript profiles). Multiple tissue-independent gene expression biomarkers were constructed from microarray experiments in which primary human hepatocytes and cancer cell lines derived from human hepatocyte, breast, and lung were exposed to chemicals (e.g., oltipraz, sulforaphane, CDDO-Im) known to activate Nrf2 or in which the Nrf2 suppressor Keap1 was knocked down. Biomarker genes were identified which had consistent directional expression changes across the comparisons and then were further filtered for those dependent on Nrf2 by removal of genes not affected by siRNA-mediated Nrf2 knockdown. The resulting 10 preliminary biomarkers were compared to 298 gene expression comparisons (biosets) from HepG2 cells or primary human hepatocytes curated in the NextBio database. The biosets spanned exposures to 55 chemicals with known Nrf2 activity based on ToxCast and Tox21 high-throughput Nrf2 assays carried out in HepG2 cells. Comparisons were carried out using the Running Fisher algorithm, a statistical test of pair-wise similarity in expression. Balanced accuracies ranged from 73-82%. The most predictive biomarker consisted of 70 genes that included many well-known Nrf2 targets (e.g., AKR1B10, AKR1C1, NQO1, TXNRD1, SXNRD1). In an examination of the biosets in the human microarray database, the biomarker was able to identify 1) many known Nrf2 chemical activators in diverse cell lines including aryl hydrocarbon receptor activators known to activate Nrf2 secondary to increases in CYP-mediated oxidative stress, 2) known environmental stressors including tobacco smoke, and 3) known (KEAP1,

NFE2L2) and novel genes that when overexpressed or knocked down affect Nrf2 activation. In summary, we provide computational methods for mining a large human microarray database to find modulators of Nrf2 that can be used to populate adverse outcome pathway networks that link oxidative stress and a number of adverse outcomes. This abstract does not represent US EPA policy

2625 Predictive Model of Systemic Toxicity

M. Martin[2], L. Pham[2], J. Clouzeau[1], S. Loisel-Joubert[1], D. Blanchet[1], H. Noçairi[1] and G. Ouedraogo[1]. *[1]L'Oréal, Paris, France and [2]NCCT, US EPA, Research Triangle Park, NC.* Sponsor: M. Martin.

In an effort to ensure chemical safety in light of regulatory advances away from reliance on animal testing, US EPA and L'Oréal have collaborated to develop a quantitative systemic toxicity prediction model. Prediction of human systemic toxicity has proved difficult and remains a gap in chemical safety assessment using alternative approaches. By leveraging multiple data sources including high-throughput screening (ToxCast HTS) and chemical descriptor and property (ToxPrint) data, a predictive model of systemic point-of-departures (POD, e.g., LOAEL or LEL) was developed. The model specifically predicts the study-level POD using data from roughly 3000 studies across nearly 600 chemicals. Systemic POD were curated in ToxRefDB from numerous study types, across multiple species and dose administration methods. Rather than attempt to adjust all POD to a single species or strain or dose duration or study type or administration method, these ToxRefDB parameters were included as covariates in the modeling process. Using Random Forest modeling, ToxRefDB covariates alone accounted for roughly 6% of the variance in the test set data. A model developed only with ToxCast HTS or ToxPrint data accounted for 44% of the variance, whereas a model developed using ToxRefDB covariates, ToxCast and ToxPrint data accounted for 53% of the variance; with a +/- 1-log confidence interval. The combination of covariates and features explain more variance in the data than either do individually, demonstrating the advantage of incorporating ToxRefDB covariates into the modeling process instead of adjusting POD a priori. The final resulting model was also enriched for features measuring xenobiotic metabolism gene expression as well oxidative stress markers demonstrating the importance for accounting for kinetics and non-specific bioactivity in predicting systemic toxicity. Herein, we have generated an externally predictive model of systemic toxicity capable of being used as a safety assessment tool. This abstract does not necessarily represent US EPA policy.

2626 Profiling and Evaluating Environmental Chemicals That Induce Oral Acute Toxicity Using Mitochondrial Membrane Disruption Assay, Big Data and New Read-Across Strategy

W. Wang[3], D. Russo[3], M. Kim[3], R. Huang[4], M. Xia[4], T. Hartung[1] and H. Zhu[3]. *[1]CAAT, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD; [2]Center for Computational and Integrative Biology, Rutgers University, Camden, NJ; [3]Department of Chemistry, Rutgers University, Camden, NJ and [4]National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD.*

Assessment of chemical animal toxicity using *in vitro* assays, especially High-Throughput Screening (HTS) assays, or *in silico* models is of great interest for alternatives to traditional animal models. However, due to the complexity of animal and human toxicity, neither *in vitro* assay nor *in silico* models have acceptable predictive accuracy to evaluate new compounds for most animal toxicity endpoints. Aiming at building a mechanism-driven *in silico* approach for animal acute toxicity, we start from a dataset of mitochondrial membrane potential disruption (MMP) quantitative High-Throughput Screening (qHTS) assay, which we combine with all publically available toxicity assay data. Firstly, we use the in-house rat oral toxicity database of 4,647 compounds and the MMP qHTS dataset of 7,320 compounds to automatically search PubChem, the largest public data sharing portal, for all *in vitro* assay data to establish bio-profiles for compounds found in both databases. Next, we use fragmental descriptors to cluster all the compounds into subsets and optimize the bio-profiles for compounds in each subset. Using a novel bio-similarity search tool developed in our lab, we are able to evaluate animal toxicants by both the most statistical significant chemical descriptors and the relevant assay testing results, to read-across and cross-validate MMP qHTS data as well as acute toxicity outcomes. This new read-across strategy will not only fill data gaps for non-tested new compounds but also be able to indicate potential toxicity mechanisms by linking the chemical and biological profiles to cellular responses and the respective hazard. The established automatic chemical *in vitro-in vivo* profiling workflow will be applicable to develop predictive models for other toxicity endpoints.

2627 The Influence of Data Curation on QSAR Modeling - Examining Issues of Quality versus Quantity of Data

A. J. Williams, K. Mansouri, A. Richard and C. Grulke. *National Center of Computational Toxicology, US EPA, Research Triangle Park, NC.*

The construction of QSAR models is critically dependent on the quality of available data. As part of our efforts to develop public platforms to provide access to predictive models, we have attempted to discriminate the influence of the quality versus quantity of data available to develop and validate QSAR models. We have focused our efforts on the widely used EPISuite software that was initially developed over two decades ago. Specific examples of quality issues for the EPISuite data include multiple records for the same chemical structure with different measured property values, inconsistency between the structure, chemical name and CAS registry number for single records, the inability to convert the SMILES strings into chemical structures, hypervalency in the chemical structures and the absence of stereochemistry for thousands of data records. Relative to the era of EPISuite development, modern cheminformatics tools allow for more advanced capabilities in terms of chemical structure representation and storage, as well as enabling automated data validation and standardization approaches to examine data quality. This presentation will review both our manual and automated approaches to examining key datasets related to the EPISuite training and test data. This includes approaches to validate between chemical structure representations (e.g. molfile and SMILES) and identifiers (chemical names and registry numbers), as well as approaches to standardize the data into QSAR-consumable formats for modeling. We have quantified and segregated the data into various quality categories to allow us to thoroughly investigate the resulting models that can be developed from these data slices and to examine to what extent efforts into the development of large high-quality datasets have the expected pay-off in terms of prediction performance. This abstract does not reflect US EPA policy.

2628 Quantitative Prediction of Skin Sensitization Potency Based on Structural Alert Spaces

M. L. Chilton, S. J. Canipa, J. Plante and J. D. Vessey. *Lhasa Limited, Leeds, United Kingdom.* Sponsor: C. Barber.

The prediction of skin sensitisation potency is demonstrated by using a similarity approach within a defined chemical space. Quantitative predictions are made by considering the similarity between compounds which contain the same structural alert, thus restricting the chemical space to those compounds which are believed to cause skin sensitisation via the same mechanism. Skin sensitisation potency can be measured using the estimated concentration of chemical required to induce a 3-fold increase in activity over the baseline in the murine local lymph node assay, a value known as the EC3. In this work the EC3 is predicted using a reference set of 465 compounds with known EC3 values which fire one, and only one, structural alert. These compounds cover 31 different structural alerts, each having a minimum of 4 compounds per alert. The reference dataset contained some compounds which fired a structural alert but were experimental non-sensitisers; these compounds were treated as having a molecular weight (MW)/EC3 value of 1, an approach which has been used by others modelling EC3 values. The methodology was evaluated using a leave-one-out cross validation. A value of MW/EC3 for each compound in the dataset was predicted by calculating the weighted mean MW/EC3 of (up to) the top 10 most similar near neighbours in the reference set that fired the same alert. The weighting for each near neighbour was derived from its structural similarity to the query compound, calculated using a Tanimoto score based on an in-house fragment-based descriptor. The performance of the model was judged based on correctly predicting EC3 values within commonly used potency categories (extreme = <0.1%, strong = ≥0.1% and <1%, moderate = ≥1% and <10%, weak = ≥10% and ≤100%). The approach proved to be conservative; 38% of the predictions fell within the correct potency category, and the model was able to assign compounds to either the correct, or next most potent, category 66% of the time. This model will be integrated into the upcoming release of Derek Nexus.

2629 Improvements to *In Silico* Skin Sensitization Predictivity After Access to Proprietary Data

D. S. Macmillan[2], S. J. Canipa[2], R. V. Williams[2], J. Hillegass[1] and J. Gould[1]. [1]*Bristol-Myers Squibb, New Brunswick, NJ and* [2]*Lhasa Limited, Leeds, United Kingdom.*

Predicting skin sensitisation potential is an important requirement for a variety of chemical safety assessments, including submissions for REACH (Registration, Evaluation, Authorisation and restriction of Chemicals), the toxicity assessment of new cosmetic ingredients, and risk assessments associated with occupational exposure. The use of animal tests (e.g. murine local lymph node assay (LLNA)) to predict this endpoint has been restricted in the EU due to the recent amendment to EU Directive 76/768/EEC. As a result, *in silico*, in chemico and *in vitro* methods are increasingly used and there is a drive to encourage data sharing from industry. *In silico* skin sensitisation alerts in Derek Nexus are built on a mixture of public and proprietary data, however, there are still gaps in the chemical space which can be covered by proprietary data. As part of a confidential on-site data sharing project with one of our members, Bristol-Myers Squibb Company (BMS), we had access to a data set of 467 active pharmaceutical ingredients and intermediates with LLNA data. The data was anonymised and the compounds analysed by clustering (a structural fingerprinting technique) and by-eye to identify unexplored chemical space. Following data analysis, 7 new alerts and modifications to 5 existing alerts were discovered, including a number of activated heterocycles, azides such as DPPA (diphenylphosphoryl azide), and epothilone analogues. Implementing these new alerts and alert modifications had a significant effect on performance of Derek Nexus against the BMS data set. Positive predictivity improved from 40% to 53% and negative predictivity improved from 82% to 89%, mainly due to the large decrease in false negatives, from 62 to 38 after data sharing. Sensitivity improved from 44% to 66% and specificity from 79% to 82%. The mechanistic and structural scope of these alerts will be presented herein and will be released in an upcoming version of Derek Nexus. Building on the success of this work, further data sharing initiatives are in progress.

2630 Translating from Preclinical In-Life Observations to Human Side Effects (eTOX III)

K. A. Briggs[2] and F. Pognan[1]. [1]*Novartis Institute For Biomedical Research, Basel, Switzerland and* [2]*Research Group, Lhasa Limited, Leeds, United Kingdom.*

Preclinical studies performed during drug development are used to identify the potential toxicity of drug candidates. A number of different finding types are collected some of which are more easily translated to effects observed in humans e.g. increased bilirubin. Other findings, including many in-life observations, are not directly translatable e.g. kinked tail but may still prove to be predictive for human side effects. We extracted preclinical in-life observations from the eTOX 2015.2.0 database. This database consists of 1,754 substances and 6,309 studies donated by 13 pharmaceutical companies in the framework of the European Innovative Medicines Initiative eTOX project. We then used chemical identifiers available in DrugBank to link this dataset to human side effects reported in the SIDER database. This resulted in a small dataset of 49 drugs for which we generated binary values for 253 preclinical in-life observations (1 - observation considered treatment related; 0 - observation either absent or considered not treatment related) and 527 human side effects (1 - side effect reported; 0 - side effect not reported). Analysis of this combined dataset (contingency table, p-value using Fisher's exact test 2- tail) revealed a number of statistically significant associations between preclinical in-life observations and human side effects. Some of these associations, e.g. ulceration in animals and haemorrhage in humans, have a possible mechanistic rationale. Others, e.g head shaking in animals and diabetic nephropathy in humans, are considered to be artefacts due to the small size of the dataset. We report on a workflow used to identify potential associations between preclinical in-life observations and human side effects.

2631 Systems Biology Investigation of Endocrine Disruptor Chemicals and Obesity

P. Ruiz[3], A. Perlina[2] and A. Feroe[1]. [1]*ATSDR/CDC, Atlanta, GA;* [2]*Sanford-Burnham Medical Research Institute, La Jolla, CA and* [3]*Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry/CDC, Atlanta, GA.* Sponsor: P. Ruiz.

The mechanisms of three endocrine disruptor chemicals (EDCs), dichlorodiphenyldichloroethylene (p,p'-DDE), bisphenol A (BPA) and tributyltin (TBT) were assessed in association with obesity using the systems biology solution suite MetaCore (Thompson Reuters). MetaCore is comprised of molecular interactions and other ontologies based on experimental evidence from manually curated full-text scientific articles. Using interactions from advanced database search queries, as well as from existing canonical MetaCore pathway maps, and subsequent directional network construction, it was possible to identify common downstream mechanisms for the suspected obesogen, p,p'-DDE, as its pathway overlaps with those of the well-established obesogens, TBT and BPA. We found that the three EDC pathways converged on three key receptors related to obesity: peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer binding proteins (C/EBPα), and estrogen receptor (ESR1). These receptors are thought to promote obesity through the known activation of adipogenic regulators, fatty acid biosynthesis, triacylglycerol metabolism, leptin signaling, glucose transport, and fat cell differentiation in adipose tissue. While the EDC structures and their direct primary targets may be different, the systems biology approach paved the downstream paths, which potentially connect these EDCs to common biological triggers implicated in obesity. The potential global network illustrates a unique systems biology model of the joint EDCs' molecular mechanisms toward promotion of obesity. This approach could guide research, lead targeted experimental testing and confirmation of the joint health effects of mixtures of EDCs.

2632 A Systematic Review of the Zebrafish Embryological Test as an Alternative to the Mammalian Developmental Test (OECD 414): A Pilot for an Evidence-Based Toxicology Approach

A. Maertens[8], R. Wright[8], M. Stephens[8], S. Hoffmann[8], C. Willett[4], H. Witters[7], F. Busquet[3], M. Lalu[6], B. Flick[1], K. Thompson[2], T. Hartung[8] and K. Tsaioun[8]. [1]*BASF, Ludwigshafen, Germany;* [2]*Bristol-Myers Squibb, New York, NY;* [3]*CAAT-EU, Konstanz, Germany;* [4]*Humane Society, Washington, DC;* [5]*Johns Hopkins University, Baltimore, MD;* [6]*Ottawa Hospital, Ottawa, ON, Canada;* [7]*Vito, Antwerp, Belgium and* [8]*Evidence-Based Toxicology Collaboration, Johns Hopkins University, Baltimore, MD.* Sponsor: K. Tsaioun.

An international collaboration of stakeholders is put together to adapt clinical medicine's evidence-based approaches based on transparency, objectivity and consistency to the unique requirements of toxicology, termed evidence-based toxicology (EBT). In the safety sciences, EBT and its core tool, systematic review (SR), will enable the integration of safety evidence in a transparent and unbiased manner to be used by regulators, the interested public and other stakeholders in assessing the safety of drugs and other chemicals and decision-amking. Another relevant application of EBT is the comparison and validation of tests methods. Here the authors, members of the collaborative work group, for the first time conduct a SR of two toxicity test methods and present the SR protocol and results of the pilot study. OECD Test Guideline 414 (Prenatal Developmental Toxicity) in small mammals is currently mandated by various regulatory regimes for chemicals registration. EU REACH legislation has created a bottleneck in testing of chemicals for registration as potential developmental hazards. This is being addressed by a few alternative approaches using different decision trees and relying on various combinations of other mammalian OECD test guidelines (TGs). Here, for the first time, systematic review is used to compare the performance of one toxicity test (the Zebrafish Embryo Test (ZET)) against a reference test (OECD TG 414). We performed a systematic review of all available literature that uses the ZET (the index test) to screen panels of chemicals, including pharmaceutical drugs, industrial and environmental chemicals. These results, after assessment and comparison for methodological performance, were then compared with the results of OECD TG 414 (comparator test). The protocol, literature and database search strategies for this SR will be presented, and the applicability of SRs to the assessment of the performance of other tests will be discussed.

2633 A New Statistical Approach to Characterize Chemical-Elicited Effects in Behavioral Data from High-Throughput Studies of Zebrafish Exposed to Diverse Chemicals

G. Zhang[1], L. Truong[2], R. L. Tanguay[2] and D. M. Reif[1]. *[1]North Carolina State University, Raleigh, NC and [2]Oregon State University, Corvallis, OR.*

Zebrafish have become an important alternative model for characterizing chemical bioactivity, partly due to the efficiency at which systematic, high-dimensional data can be generated. However, these new data present analytical challenges associated with scale and diversity. We develop a novel, robust statistical approach to characterize chemical-elicited effects in behavioral data from high-throughput screening (HTS) of all 1060 ToxCast chemicals across 5 concentrations at 120 hours post-fertilization (hpf). Taking advantage of the immense scale of data for a global view, we show that this new approach reduces bias introduced by extreme values yet allows for diverse response patterns that confound the application of traditional statistics. We also show that, as a summary measure of response for local tests of chemical-associated behavioral effects, it achieves an 80% reduction in coefficient of variation. This effective increase in signal-to-noise ratio augments statistical power and is observed across experimental periods (light/dark conditions) that display varied distributional response patterns. Finally, we integrate results with data from concomitant developmental endpoint measurements to show that appropriate statistical handling of HTS behavioral data can add important biological context that informs mechanistic hypotheses.

2634 Handling Missing Data in Chemical Prioritization and Bioactivity Profiling Applications Using ToxPi

K. To[1], A. Nguyen[2], R. C. Fry[2] and D. M. Reif[1]. *[1]Bioinformatics Research Center, North Carolina State University, Raleigh, NC and [2]Gillings School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

The Toxicological Priority Index (ToxPi) is a method for prioritization and profiling of chemicals that integrates data from diverse sources. However, individual data sources ("assays"), such as *in vitro* bioassays or *in vivo* study endpoints, often feature sections of missing data, wherein subsets of chemicals have not been tested in all assays. In order to investigate the effects of missing data and recommend solutions, we designed simulation studies around high-throughput screening data generated by the ToxCast and Tox21 programs on chemicals highlighted by the Agency for Toxic Substances and Disease Registry's (ATSDR) Substance Priority List (SPL), which helps prioritize environmental research and remediation resources. Our simulations explored a wide range of scenarios concerning data (0-80% assay data missing per chemical), modeling (ToxPi models containing from 160-700 different assays), and imputation method (k-Nearest-Neighbor, Max, Mean, Min, and Binomial). We compared scenarios by estimating the RMSE between ToxPi profile scores, as well as recording the mean change in priority rank. We found that the choice of imputation strategy exerted significant influence over both scores and associated ranks, and the most sensitive scenarios were those involving fewer assays plus higher proportions of missing data. By characterizing the effects of missing data and the relative benefit of imputation approaches across real-world data scenarios, we can augment confidence in the robustness of decisions made regarding SPL chemicals.

2635 Using "Big Data" to Identify Model Compounds and Develop Adverse Outcome Pathways for Mitochondrial Dysfunction

D. Dreier, N. Denslow and C. Martyniuk. *University of Florida, Gainesville, FL.*

Adverse outcome pathways (AOPs) provide a conceptual framework for toxicology research and risk assessment, associating molecular initiation events (MIEs) and toxicity pathways to higher-level biological effects such as impaired metabolism or reproduction. In order to develop AOPs, it is important to identify compounds that induce changes in specific MIEs. For example, AOPs for reproductive dysfunction in fish and mammals have been developed for estrogen receptor antagonism, androgen receptor agonism, and aromatase enzyme inhibition. However, AOPs for mitochondrial dysfunction and oxidative stress across species have not been fully established for reproduction. Recent efforts in high-throughput screening (e.g., ToxCast) have identified chemicals that cause mitochondrial dysfunction and oxidative stress. Thus, the objective of this study was to leverage *in silico* analyses of "big data" to identify compounds that act specifically through these toxicity pathways for AOP development. Assays related to mitochondrial dysfunction, oxidative stress, and endocrine disruption were compiled for Phase I and II compounds from ToxCast, transformed into nonparametric ranks, and imported into ToxPi. Compounds were further ranked by comparing ToxPi slice values for mitochondrial dysfunction and oxidative stress relative to endocrine signaling pathways. The analysis determined that many endocrine disrupting compounds, particularly androgen receptor agonists, are also likely to result in mitochondrial dysfunction and oxidative stress. Furthermore, nitrophenol, anthraquinone, and select chlorophenol compounds were those chemicals most likely to impact mitochondrial function while also having limited effects on endocrine signaling. These chemicals are top candidates for developing non-endocrine mediated AOPs, and this study presents a novel data-mining and computational approach to identify compounds that cause specific toxicity pathways.

2636 Zebrafish High-Throughput Data Analysis and Quality Control Pipeline

S. Marvel[1], L. Truong[2], R. L. Tanguay[2] and D. M. Reif[1]. *[1]Bioinformatics Research Center, North Carolina State University, Raleigh, NC and [2]Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR.*

High-throughput screening of chemical bioactivity using embryonic zebrafish has become a valuable platform for characterizing chemical hazard by providing rapid, systematically-generated data on both morphological endpoints and behavioral responses. As with any high-dimensional data generated at sufficiently large volumes, slight artifacts may affect both global and local conclusions. Here, we address detection and remediation of the key Quality Control (QC) concepts of uniformity and outlier detection. In the context of continuous measures of behavior (e.g. movement), uniformity refers to the evenness of a response surface across plate/well locations, experiments, or other physical parameters, and outlier detection refers to movement values believed to be unrealistic. We have developed novel approaches to detect and account for both: uniformity is improved by performing a distributional transformation to remove the association between movement values and plate location; outlier detection removes spurious values by comparing movement values to a characteristic distribution based upon subsets of the data. Thus, the same Big Data that allow detection of problems can be harnessed to remediate those issues and, ultimately, provide quantifiable metrics for QC that lend greater confidence in data fidelity than would be possible without global context. We have developed a graphical user interface (GUI) that includes these important QC methods alongside a full suite of statistical methods, providing efficient integration of all analysis phases.

2637 Modeling Steroidogenesis Disruption Using High-Throughput *In Vitro* Screening Data

A. L. Karmaus[1] and M. T. Martin[2]. *[1]ORISE - US EPA/ORD/NCCT, Durham, NC and [2]US EPA/ORD/NCCT, Durham, NC.*

Environmental chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis) causing adverse reproductive and developmental effects. Historically, a lack of assays resulted in few chemicals having been evaluated for effects on steroidogenesis. The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes, yet the only enzyme in the pathway for which a high-throughput screening (HTS) assay has been developed is aromatase (CYP19A1), responsible for the aromatization of androgens to estrogens. Recently, the ToxCast HTS program adapted the OECD validated H295R steroidogenesis assay using human adrenocortical carcinoma cells into a high-throughput model to quantitatively assess the concentration-dependent (0.003-100 μM) effects of chemicals on 10 steroid hormones including progestagens, androgens, estrogens and glucocorticoids. These results, in combination with two CYP19A1 inhibition assays, comprise a large dataset amenable to clustering approaches supporting the identification and characterization of putative mechanisms of action (pMOA) for steroidogenesis disruption. In total, 514 chemicals were tested in all CYP19A1 and steroidogenesis assays. 216 chemicals were identified as CYP19A1 inhibitors in at least one CYP19A1 assay. 208 of these chemicals also altered hormone levels in the H295R assay, suggesting 96% sensitivity in the ability of hormone quantification to identify CYP19A1 inhibitors. Interestingly, only 18 of these chemicals were identified in both CYP19A1 assays, 17 were also identified in the steroidogenesis assay. Of these 17 chemicals, all elicited effects on estrogen levels and 13 had effects on androgen levels, as expected with CYP19A1 inhibition. The steroidogenesis assay data were also used to

model CYP17A1 inhibition. We suggest that increases in progestagens and glucocorticoids with simultaneous decreases in the levels of androgens and estrogens comprise a profile indicative of CYP17A1 inhibition, which correctly identified known CYP17A1 inhibitors such as conazole fungicides including prochloraz. Cumulatively, our examples demonstrate that computational profiling of *in vitro* data provides a model that characterizes pMOAs for steroidogenesis disruption to support the identification of endocrine disruptive chemicals. This abstract does not necessarily reflect US EPA policy.

PS 2638 Electronic Cigarettes: Users' Flavor Preferences and Associated Health Effects

M. Hua and P. Talbot. *Department of Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA; Environmental Toxicology Graduate Program, University of California, Riverside, Riverside, CA.*

We previously showed that some chemical flavorings in electronic cigarettes (EC) are cytotoxic. To study the relationship between EC flavors and health effects, we surveyed 2,753 respondents from UC Riverside and online health forums. Of 2,753 survey takers, 835 were EC users between ages 18-22 (49.46%), male (71.74%), and listed 'some college' as highest education (38.92%). Most represented ethic groups were White/Caucasian (43.23%), Asian (23.38%), and Hispanic/Latino (18.92%). Many users smoked/tried cigarettes (86.93%) but no longer smoke (67.95%), listing EC (53.06%) and nicotine replacement products as aids to quit smoking (48.98%). EC use was 'influenced by friends' (54.34%) and 'vaping is safer than smoking' (55.33%). Most users described their health as 'very good/good' (72.70%) and vaped daily (81.57%) for at least 1 month to 2 years (73.62%). EC refill fluids (ECRF) deemed flavors 'very important' (50.95%)/'important' (27.12%) in users' decision to vape. The most popular flavors for 789 respondents who used ECRF: were Berries/Citrus [n=559 (70.85%)]; Sweets [n=406 (51.46%)]; and Bakery [n=321(40.68%)]. ECRF flavors in 17 categories were associated both positive (n=205) and negative (n=116) health effects in 10 body systems. Most positive effects were reported for the Respiratory (RSP) (n=144), Neurological (n=100) and Cardiovascular (CV) systems (n=62), and related to 'Mixed Combo', 'Tobacco', and 'Sour' flavors. Most negative effects were reported in RSP (n=111), CV (n=72), and EENT systems (n=28), and related to 'Aromatic', 'Buttery/Caramel/Vanilla', and 'Menthol' flavors. Users also perceived positive (n=175) or worsened (n=13) pre-existing symptoms linked to EC. Positive effects on pre-existing conditions were reported for RSP (51.69%), EENT (29.21%), and NL/skin (anxiety/depression) (18.29%) systems. For negative effects, systems most affected were RSP (46.15%), and EENT/skin (7.69%). Positive/negative effects related to pre-existing symptoms were: coughing, perception of smell, and skin clarity. Perceptions concerning the health effects associated with EC and/or EC flavors are varied and some health effects are linked by users to flavors.

PS 2639 Prediction of Clastogen, Aneugen, and Non-Genotoxicant Group Membership Using an *In Vitro* Multiplexed Flow Cytometric Assay and Multinomial Logistic Regression

S. Bryce, D. Bernacki, J. C. Bemis and S. Dertinger. *Litron Laboratories, Rochester, NY.*

Individual biomarkers that were found to have predictive value for classifying agents as clastogenic, aneugenic, or non-genotoxic were multiplexed into a miniaturized, add and read-type flow cytometric assay. Reagents included a detergent to liberate nuclei, propidium iodide and RNase to serve as a pan-DNA dye, fluorescent antibodies against γH2AX, phospho-H3, and p53, and fluorescent microspheres for absolute nuclei counts. Experiments were conducted with 48 reference chemicals using TK6 cells. Exposure occurred for 24 hrs in 96 well plates, and unless precipitation or foreknowledge about cytotoxicity suggested otherwise, the highest concentration was 1 mM. At 4 and 24 hrs, cell aliquots were removed and added to wells of a microtiter plate containing the working reagent mix. After a brief incubation period robotic sampling was employed for walk-away flow cytometric data acquisition. A forward stepping approach was used to build multinomial logistic regression models and showed that four endpoints combine for high predictivity in terms of group membership: 4hr γH2AX and phospho-H3 values, and 24 hr p53 and polyploidy values. Weighting the model on cytotoxicity (relative nuclei counts at 24 hrs) resulted in higher ROC and pseudo R2 values. Cross validation of the weighted, four-factor model occurred via a leave-one-out approach, and 91% concordance with our a priori classifications was observed. These initial results are encouraging as they suggest a supervised machine learning platform can reliably predict new chemicals' group membership based on data from an efficient and highly scalable multiplexed assay. More work is planned with a broader range of chemicals, additional treatment schedules and cell lines, and other laboratories in order to further evaluate the merits and limitations of this approach.

PS 2640 ToxCast Profiling in a Human Stem Cell Assay for Developmental Toxicity

T. Knudsen, P. Kothiya and K. Houck. *National Center for Computational Toxicology, US EPA, Research Triangle Park, NC.*

We correlated the ToxCast library in a metabolic biomarker-based *in vitro* assay (Stemina devTOXqP) utilizing human embryonic stem (hES) cells (H9 line). This assay identifies the concentration of a chemical that disrupts cellular metabolism in a manner indicative of teratogenicity [Palmer et al. 2013]. Undifferentiated H9 cells were exposed for 72h and media from the final 24h period was analyzed by LC-MS to determine the ornithine to cystine ratio (ORN/CYSS). ORN is derived from arginine breakdown during the citric acid cycle and CYSS is formed by oxidation of cysteine molecules that covalently link via a disulfide bond, and the corresponding 'teratogen index' based on ORN/CYSS falling below 0.88. To date, the raw and plate-normalized data for 286 samples in concentration series (269 chemicals plus replicates, n=3) and another 812 samples at a single concentration (n=4) have been entered into the ToxCast pipeline for QA, processing, analysis and eventual release to the public. A preliminary analysis revealed the following trends. First, 166 compounds (15.5% of the tested compounds) were 'active' based on the default ORN/CYSS threshold (0.88). These included many known teratogens, such as trans-retinoic acid (LEC = 3 nM), 5-fluorouracil (100 nM), methotrexate (100 nM), thalidomide (300 nM), and carbamazepine (3 uM) among others. Second, for 23 compounds with FDA codes (A,B,C,D,X) or ECVAM classifiers, the default model had a balanced accuracy of 84% (sensitivity 0.80, specificity 0.88). Third, many chemicals not yet classified were predicted positive, including the angiogenesis inhibitors TNP-470 (10 nM) and 5HPP-33 (10 uM). Fourth, at the concentrations tested specificity was demonstrated for a parent-metabolite pair where only the proximate teratogen was active; for chemical stereoisomer pairs where only one compound was active; and for 3 closely-related structural isomers where only one structure was active. Cross-referencing with ToxCastDB (*in vitro*) and ToxRefDB (*in vivo*) is being undertaken to assess the added value of the devTOXqP assay performance in computational models built for predictive teratogenicity in a human cell-based system. (Disclaimer: this abstract does not reflect EPA policy).

PS 2641 Integrating Animal Data from the Past with Future CDISC-SEND Compliant Data (eTOX VI)

M. Cases[4], K. Briggs[2], W. Muster[1], A. Brigo[1] and P. Marc[3]. *[1]F. Hoffmann-La Roche AG, Basel, Switzerland; [2]Lhasa Limited, Leeds, United Kingdom; [3]Novartis Institutes for BioMedical Research, Basel, Switzerland and [4]Genetic Toxicology, Bayer Pharma AG, Berlin, Germany.*

The eTOX database was started in 2010 as an effort to integrate legacy toxicology information from the 13 pharmaceutical partners contributing to the project. Data from animal studies have been historically hard to compare to human data because of the diversity of archiving formats and vocabulary used. A lot of eTOX efforts are focused on the collection and normalization of the information content for over 6,000 study records. From ending 2016 on, nonclinical data relevant for drug submissions to the FDA will have to be compliant with the CDISC Standard for Exchange of Nonclinical Data (SEND). It will enforce the use of both the standard format and terminology harmonization and it will offer an ideal platform for data sharing and cross studies analysis. The emergence of this standard raised the question of the inter-operability between eTOX, containing retrospective data; and SEND, the prospective data vehicle of the future. Here we present the processes we developed the last few years to map terms to the standards, including our open-source solution named OntoBrowser. We will also discuss how we constructed a process able to load data generated as SEND packages into the eTOX database, and the key challenges we faced. Additionally, we will discuss how retrospective data contained in the eTOX database can influence the future standards

2642 Why Are Most PLD Active Chemicals Also hERG Active?

R. D. Beger[1], S. Slavov[1], R. Huang[2] and M. Xia[2]. *[1]NCTR, US FDA, Jefferson, AR and [2]NIH NCATS, Rockville, MD.*

Datasets of 4096 compounds assayed for human Ether-à-go-go Related Gene hERG blocking and 5490 compounds assayed for phospholipidosis (PLD) induction were used to test a hypothesis that both phenomena are driven by similar structural features. To eliminate the chemical domain effect, a filtered dataset of 572 compounds tested in both bioassays was constructed. Of these, 109 compounds were PLD+, while 463 were PLD-. On the hERG side 236 were hERG+, while 336 were hERG-. A 3D-SDAR model was developed using 13C and 15N NMR spectra. The C-C region was binned using a 10ppm x 10ppm x 0.5Å grid, while the C-N region was binned using a 10ppm x 25ppm x 0.5Å grid and the N-N region using a 25ppm x 25ppm x 0.5Å grid. For each endpoint a total of 100 randomized training/hold-out test set SDAR models were generated. The hold-out test set performance characteristics for hERG were 0.89 for accuracy, 0.83 for sensitivity and 0.93 for specificity, while the hold-out test set performance characteristics for PLD were 0.78 for accuracy, 0.89 for sensitivity, and 0.76 for specificity. 3D-SDAR maps of the most frequently occurring bins suggested 3-center toxicophores composed of two aromatic rings and an amino-group for both hERG and PLD. U-tests on the original 3D-SDAR bins indicated that the main difference between the hERG and PLD toxicophores is in the distance between the two aromatic rings. In case of hERG this distance varied from 4Å to about 11.5Å, while for PLD the upper bound was at about 7.5Å. In both cases the distances between the aromatic rings and the amino group varied from about 5Å to about 10Å. These results might explain an earlier observation (Sun et al Bioorg. Med. Chem. Lett. 2013, 23, 4587), that most PLD inducers are also hERG blockers, while fewer hERG inhibitors are PLD actives. Additional sets of 1823 (hERG) and 1167 (PLD) compounds, which were not assayed at the time of model building, were selected to evaluate the true external predictive power of the models.

2643 Chemical Risk Analysis with Bayesian Support (CRABS) in the IVIVE Context

L. Burgoon. *US Army Engineer Research and Development Center, APEX, NC.*

The context of the chemical challenge drives the types of risk-based decisions that must be made in chemical safety policy. For instance, environmental risk managers make different decisions (e.g., site cleanup, post hoc exposure/emissions control) when compared to their chemical manufacturing risk management counterparts. However, the goals of chemical risk managers across these fields remain the same: limit chemical-induced disease/injury. I propose that Bayesian Decision Theory can be a unifying foundation for both environmental chemical and chemical manufacturing risk managers when considering *In Vitro* to *In Vivo* Extrapolation (IVIVE). Under the proposed Chemical Risk Analysis with Bayesian Support (CRABS) approach, risk management decisions are made based on minimizing the risk (and thus the cost) of a decision under uncertainty using Bayes theorem. Under CRABS, the severity scores for different diseases from the Global Burden of Disease (GBD) study are used to weight chemical-induced diseases, and thus chemicals, in combination with chemical risk information. CRABS calculates the risk of a chemical resulting in a disease based on the risk of disease given a chemical's dose/concentration and the probability of a chemical exposure. Risk managers then use CRABS to choose the decision rule that minimizes the risk adjusted for disease severity from the GBD. This will result in science and disease based policies that will address chemical prioritization, policy-based risk management and global stewardship needs. I will demonstrate how CRABS and the AOPXplorer can work together to facilitate the extrapolation from *in vitro* to *in vivo* to facilitate risk decisions.

2644 Cramer Class Determination and Its Role in the Safety Assessment of Fragrance Materials

J. Shen[1], A. M. Api[1], D. W. Roberts[4], A. Aptula[3] and T. W. Schultz[2]. *[1]Research Institute for Fragrance Materials, Woodcliff Lake, NJ; [2]College of Veterinary, The University of Tennessee, Knoxville, TN; [3]Safety and Environmental Assurance Centre, Unilever, Bedford, United Kingdom and [4]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom.*

The Cramer decision tree classifies organic chemicals into one of three classes reflecting the probability of low, moderate and high toxicity in an explicit way. It has been extended to the structure-based Threshold of Toxicological Concern (TTC) and is used by Research Institute for Fragrance Materials for assessing fragrance materials safety without the need for generating data. Therefore, assigning the appropriate Cramer class to a chemical that lacks toxicity data is a crucial step to ensure the integrity of its risk assessment. Usually, freely available *in silico* tools, such as Toxtree and OECD QSAR Toolbox and Toxtree, are used to determine Cramer class. By comparing the assignment of these *in silico* tools for ~2500 fragrance materials, 20% were identified to have discrepancy. Therefore, human expert judgement were brought in to assign Cramer class for all the fragrance ingredients. Based on the analysis of a large data set of over 2500 fragrance ingredients, we found that most of the 33 questions in the original Cramer scheme are straightforward. Through repeated examination each of the 33 questions, we found 14 where the logic underlying the development of the rule is unclear (i.e., Q1, Q9, Q11, Q16, Q17, Q18, Q20, Q22, Q24, Q25, Q26, Q29, Q30, and Q32). These questions are well served by minor wording changes and/or further explanation designed to capture what we perceive to be the intent of the original decision tree. The findings reported here could be used as guidance for conducting Cramer classification and provide advice for the improvement of the *in silico* tools.

2645 Integration of Tox21 and CTD Data: Elucidating Correlations between High-Throughput Chemical Screening and Curated Literature

G. Collier, A. Planchart, D. M. Reif and C. J. Mattingly. *North Carolina State University, Raleigh, NC.*

Initial applications of the collaborative Tox21 high-throughput screening (HTS) initiative have focused on predictive capabilities through prioritization and profiling along narrow toxicological axes or mapping assay results onto canonical biochemical or physiological pathways. There is, however, an urgent need for integration with the disease and exposure context facilitated by resources like the Comparative Toxicogenomics Database (CTD), which draws upon biomedical literature to infer chemical-gene, chemical-disease and gene-disease interactions. The current Tox21 HTS dataset has been integrated with CTD by cross-mapping shared features, such as relationships between chemicals and gene targets. Capitalizing on this similarity between datasets, we have established evidence-based connections between individual screening assays and environmental health outcomes. Integration of target-specific, mechanism-based, biological observations with manually curated data from the peer-reviewed scientific literature resulted in a unified, searchable data system where screening assay results serve to corroborate interactions reported in the literature. Integration of these datasets also demonstrates how data acquired through curated literature can lend a human health context to the Tox21 HTS results and, reciprocally, how Tox21 can address important data gaps in the literature.

2646 Mechanistic Model-Based Approach to Skin Sensitisation Risk Assessment

G. Maxwell[2], R. Cubberley[2], S. Dhadra[2], N. Gellatly[2], J. P. Gosling[1], R. Pendlington[2], J. Pickles[2], J. Reynolds[2], R. Stark[2], D. Tang[2] and C. MacKay[2]. *[1]School of Mathematics, University of Leeds, Leeds, United Kingdom and [2]SEAC, Unilever, Bedford, United Kingdom.* Sponsor: P. Carmichael.

Despite our understanding of the key events that drive skin sensitisation our ability to combine non-animal hazard data with exposure information to establish a safe level of human exposure for a sensitising chemical remains a key gap. Our aim is to apply mechanistic understanding of skin sensitisation to improve our ability to make risk assessment decisions. Central to our approach is a toxicokinetic-toxicodynamic (TKTD), ordinary differential equation (ODE) model covering several of the key events captured in the skin sensitisation adverse outcome pathway (AOP) that outputs naïve CD8+ T cell activation as a surrogate measure for sensitisation induction in humans. Chemical-specific model parame-

ters are derived from bespoke *in vitro* experiments designed to measure reactivity rate and skin bioavailability, whilst biological parameters are taken from the immunological literature. The model has been used to simulate a study published previously by Friedmann et al. in which 132 healthy volunteers were exposed to one of five doses of the contact allergen 2,4-dinitrochlorobenzene (DNCB). As a significant proportion of each dose cohort were sensitised to DNCB within this study, comparison of model simulation results to these clinical data have provided an opportunity to explore the relationship between naïve CD8+ T cell activation and clinical sensitisation. To do so, the model was parameterised for DNCB and prediction made for extent of naïve CD8+ T cell activation occurring across dose. Reverse dosimetry analysis was then performed to calculate the dose-threshold for sensitisation to DNCB and the probability of the average individual acquiring contact allergy at any given dose. Importantly, uncertainty due to both parameter uncertainty (limitations in our knowledge of parameter values) and model uncertainty (limitations due to validity of modelling assumptions) has been characterised. The uncertainty in model predictions for the DNCB case study is significant, therefore additional historical case studies are underway to address this finding.

PS 2647 Cardiosafety *In Silico* Prediction — Validation Results of a Multiscale Simulation Model (eTOX VII)

A. Amberg[3], L. Anger[3], H.-P. Spirkl[3], J.-M. Guillon[4], V. Ballet[4], F. Schmidt[3], M. Stolte[3], A. Czich[3], H. Matter[2], J. Gomis-Tena[5], L. Rodriguez[5], J. Saiz[5] and M. Pastor[1]. *[1]IMIM, GRIB, Barcelona, Spain; [2]Sanofi, LGCR, Frankfurt, Germany; [3]Sanofi, Preclinical Safety, Frankfurt, Germany; [4]Sanofi, Preclinical Safety, Paris, France and [5]Universitat Politècnica de València, Ci2B, València, Spain*. Sponsor: D. Keller.

A new paradigm to assess the proarrhythmic potential of drugs is proposed by the CiPA (Comprehensive *in vitro* Pro-arrhythmia Assay) initiative combining a suite of a priori *in vitro* assays (7 most important ion channels for cardiac activity) coupled to *in silico* reconstructions of cellular cardiac action potential (AP). The eTOX consortium has developed a multiscale simulation *in silico* model based on O'Hara/Rudy incorporating the principles of this new paradigm. The core model simulates the effects of drugs on a virtual cardiac tissue composed by different types of cardiomyocytes. The input of this model, the blockade of a set of 3 ion channels (IKr/hERG, IKs, ICaL), can be obtained experimentally or predicted using advanced 3D-QSAR models. The system predicts the % change of the QT interval at different drug concentrations in order to facilitate risk assessment. This *in silico* model was validated using Purkinje fiber assay results (input: AP prolongation and arrhythmogenic risk assessed by early after-depolarisation occurrence) from 500 in-house drug candidates. The validation showed that predictivity is highly dependent on the model's applicability domain (AD): for some chemical series the proarrhythmic potential could not be identified, for others, however, most of the positive drugs were correctly predicted with sensitivities up to 80-90% (average prediction accuracy was 70%). Retraining of this model with additional internal data should help to improve the model AD and predictivity. It is important to note that AP prolongation was correctly predicted for many proarrhythmic drugs with only low (> 30 µM) *in vitro* hERG inhibition. Furthermore, the model showed high additional benefit for read-across within a chemical series to predict the proarrhythmic potential of drugs with low solubility for which no reliable Purkinje fiber results could be obtained. These validation results showed that this cardiosafety *in silico* model can successfully be applied in R&D to predict the proarrhythmic potential of drug candidates within the model AD.

PS 2648 Development and Application of a Novel QSAR to Assess Potential Species Differences in Developmental Toxicity

H. M. Kung and J. E. Foreman. *ExxonMobil Biomedical Sciences, Inc., Annandale, NJ.*

Background: Under global chemical management schemes, the potential hazards of new chemicals must be assessed. Such an assessment can entail animal testing or, when justified, non-animal testing and alternative approaches, including read across and QSAR. [Objective] To meet regulatory requirements and to achieve the principles of the Refinement, Reduction and Replacement of laboratory animals (3Rs), a Weight of Evidence (WoE) approach was taken to assess the potential developmental hazards of a C30 branched alkane substance. Materials and methods: Using a basic toxicity dataset on the substance, a category was defined allowing for the interpolative read across to existing developmental toxicity data on analogs. Within this category, cross-species comparative toxicokinetics and systemic toxicity was assessed. Finally, a QSAR model was developed from the publicly available results of >300 developmental toxicity studies conducted in multiple species to estimate the result for a study conducted in a rabbit, which was then compared to quantitative exposure estimates to determine potential risk. Results: Within this category, the potential developmental hazards in rats were determined to be very low based on interpolative read across. Further, assessment of the chemical properties, along with systemic toxicity and toxicokinetics data from category members in multiple species led to the hypothesis that the potential developmental toxicity hazards were likely to be consistent between rats and other species, including rabbits and humans. The QSAR model data supported this hypothesis. Finally, integrating the newly-created and externally validated QSAR model results with quantitative exposure estimates, it was determined that there was minimal risk to the developing fetus and therefore a study in a second species was not considered necessary. Conclusion: Considering the full Weight of the Evidence, we therefore concluded that the potential hazards of the C30 alkane are sufficiently characterized, there is minimal risk and therefore, the conduct of a new developmental toxicity study on the C30 alkane is not scientifically justified.

PS 2649 Estimating Compound Potency in Tox21 Phase II Estrogen Receptor Assays Using Weighted Information Gain

K. R. Shockley. *NIEHS, Research Triangle Park, NC.* Sponsor: R. Tice.

Thousands of concentration-response profiles are generated by quantitative high-throughput screening experiments in phase II of the Tox21 collaboration. Estimating compound potency in these studies is important in order to directly compare compound activities or to perform downstream analyses such as prediction modeling. Most potency measures are based on the concentration producing 50% of the maximal activity using the AC50 parameter from the Hill equation model. However, AC50 estimates can accompany very large uncertainties and may not be reproducible from one experiment to the next. We propose an alternative metric based on the maximal rate of weighted information gain. Our potency measure, termed [WRIG]max, uses all the data in a concentration-response profile for estimation and can be applied to non-monotonic response patterns . We describe the calculation of the new potency measure and use the approach to estimate [WRIG]max in experimental 15-point concentration-response data obtained from Tox21 Phase II estrogen receptor agonist and antagonist assays. We found that screens performed in agonist mode showed higher correlation between experimental replicates (Pearson's r ~ 0.7) than experiments performed in antagonist mode (Pearson's r ~ 0.2 - 0.4). Statistical assessments indicate that potency differences of approximately 5-fold (agonist mode) and 10-fold (antagonist mode) could be reliably determined in the data set. Using the approach described here, the most potent compounds in the screen were associated with known hormone metabolic pathways.

PS 2650 Analysis of the Potential of Chemicals to Induce Anemia Based on Compound Specific Properties

I. Tluczkiewicz[3], M. Batke[3], S. J. Webb[4], R. Garcia-Sterna[1], P. Bento[2] and S. E. Escher[3]. *[1]Chemotargets, Barcelona, Spain; [2]European Bioinformatics Institute (EMBL-EBI), Cambridge, United Kingdom; [3]Fraunhofer ITEM, Hannover, Germany and [4]Lhasa Limited, Leeds, United Kingdom*. Sponsor: C. Dasenbrock.

For the purpose of developing new *in silico* predictive models, data sharing between many institutions of Europe was performed within the eTOX project (www.e-tox.net) and a toxicological database (DB) eTox was built. In this context, the aim of our study was to generate a model which is able to distinguish active and inactive chemicals for inducing anemia by analyzing oral subacute to chronic repeated-dose toxicity studies in rodents of the eTox, Fraunhofer RepDose and TG-GATEs DBs. The following criteria were considered to be relevant to identify hemolytic compounds: Changes of hematological parameters were considered to be mandatory e.g. a decrease in the amount of red blood cells or hemoglobin in the blood because of a decrease in RBC production or an increase in RBC breakdown. Additionally, due to the break-down of hemoglobin during hemolysis, bilirubin level and iron (hemosiderosis) should be increased in serum. Further histopathological effects in bone marrow, spleen and/or liver e.g. changes in hematopoiesis were considered. Three datasets were distinguished: Chemicals being positive *in vivo* (N= 212), inconclusive (N= 210) and negative *in vivo* (N= 806) for anemia. Subsequently emerging pattern mining was performed to extract structural fragments from the dataset that predominate in the active set. The aromatic amines, nitrobenzenes and aliphatic ethers were frequently present in the positive dataset and are well known chemi-

cals to cause hemolytic anemia by formation of methemoglobin and/or hydroxyl radical which often leads to hemolysis. Furthermore, the structural differences between active and inactive chemicals as well as the impact of other parameters (limited scope of examination, impact of study duration) on the chemical potency to induce anemia are shown.

2651 Development and Application of a Reference Database for Androgen Receptor Activity

N. Kleinstreuer[2], P. Ceger[2], P. Browne[1], D. Allen[2], J. Hamm[2] and W. Casey[2]. *[1]US EPA/OSCP, Washington, DC and [2]NIEHS/NICEATM, Durham, NC; [3]ILS, Inc., NIEHS/NICEATM, Durham, NC.*

Mandated testing of thousands of chemicals to identify those that may act as androgen receptor (AR) agonists or antagonists will cost millions of dollars and take decades to complete using current validated methods. Alternative methods using high-throughput screening (HTS) and computational toxicology technologies, such as those developed in the ToxCast/Tox21 screening programs, can rapidly and inexpensively identify potential androgen-active chemicals. Evaluation of such alternative testing strategies requires high-quality *in vitro* and *in vivo* reference data. Here, we describe a literature search for reference data from *in vitro* AR binding and transactivation (TA) assays. A list of 127 putative androgen-active or inactive chemicals was compiled based on international AR test method validation efforts. We conducted semi-automated literature reviews for *in vitro* AR binding and TA assays on these chemicals, and extracted data from identified references into a single database. Detailed assay information and results were recorded using a standardized ontology. Data were analyzed for reproducibility and species specificity. Antagonist data were only considered if they included concurrent cytotoxicity evaluations (noted in 55% of assays). Based on quantitative data such as relative binding affinity and TA activity (or inhibitory) concentrations, chemicals with reproducible results were assigned potency ranges. The reference data were used to evaluate performance of nine HTS ToxCast/Tox21 assays that map to the AR pathway and an associated computational model integrating the assays. Reference chemical lists and supporting data will be made available to the public and submitted to OECD via the Validation Management Group-Non-Animal to facilitate international harmonization of AR test method evaluations. This work does not reflect EPA policy. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

2652 Virtual Screening for Estrogen Active Chemicals in a Pharmaceutical Database Using a Novel Pharmacophore Paired Docking Search Method

G. Guirard, T. Mitchell, P. Ma and T. Wiese. *College of Pharmacy, Xavier University of Louisiana, New Orleans, LA.* Sponsor: C. Miller.

The application of virtual screening methods to identify estrogenic chemicals in databases of commercially available chemicals continues to be a challenge. We test the hypothesis that pharmacophore models coupled with ligand-receptor docking can identify estrogen active substances in a database of pharmaceutical chemicals. Of the many Estrogen Receptor alpha Ligand Binding Domain (ERLBD) crystal structure complexes in the RCSB Protein Databank, most contain novel ligands. Taken together, all of these ERLBD ligand complexes represent a wide range of ligand-receptor interactions and binding pocket configurations. Thus, there is the potential to use each ERLBD complex as a unique pharmacophore model along with docking for virtual screening. Such a method should have higher reliability than docking alone since all of the experimentally determined ER ligand binding modes would be described from both ligand and receptor points of view. In this study, multiple ERLBD crystal structure complexes containing novel ligands were each used to generate pharmacophore models that were then coupled sequentially with docking to the corresponding crystal structure in a virtual screening method. The NCGC Pharmaceutical Database (NCP) from the NIH NCATS was filtered by size and washed using MOE (Chemical Computing Group). Reasonable 3D structures were then generated with Corina (Molecular Networks) and optimized in MOE (MMFF94). Screening this NCP subset database with the Pharmacophore-Dock method using MOE produced hits that were then sorted by score. Estrogen agonist and antagonist drugs were identified (positive controls) as well as compounds with unknown estrogen activity. Hits with estrogen potential were sorted by relevance (current use pharmaceuticals or components) and when available, were obtained for validation testing in cell-based bioassays. The virtual screening process applied in this study appears to have utility in identifying estrogenic chemicals in large databases.

2653 Challenges and Lessons Learned in Developing an AOP for Mutagenic Mode of Action for Hepatocellular Carcinoma (HCC)

M. M. Moore[3], R. Schoeny[1] and L. H. Pottenger[2]. *[1]Consultant, Washington, DC; [2]Olin, Midland, MI and [3]Ramboll Environ, Little Rock, AR.*

Adverse Outcome Pathways (AOPs) provide a framework to describe a sequence of key events (KEs), starting with a molecular initiating event (MIE), followed by a series of KEs linked to one another by KE Relationships (KERs), ultimately resulting in a specific adverse outcome. Each KE/KER is supported by data and evaluated against criteria to assess biological plausibility, weight/strength of evidence, specificity, and confidence. AOPs offer an approach to apply toxicological data and use of mode-of-action (MOA) information for such purposes as read-across, integrated approaches to testing & assessment, and risk assessment. Developing an AOP on mutagenic MOA for HCC, under the OECD program, and using Aflatoxin B1 (AFB1) data, led to identification of a number of challenges as well as lessons learned. AFB1 was selected because of its rich database and general agreement that it induces HCC via a DNA-reactive MOA in many species, including humans. However, in the process of assessing the AFB1 information, it became clear that the data are, in fact, not optimal and for some of the KEs, the definitive data are not available. This realization provided both the need and the opportunity to articulate a prioritized list for the utility of specific types of data to define a mutagenic MOA. It also demonstrated the need to clarify that, although the influential KE that distinguishes a mutagenic MOA from a non-mutagenic MOA is the ability of the chemical to induce, directly and early, gene mutations in a tumor-critical gene, it is not sufficient just to determine that a chemical can induce mutations in any one of a number of mutation assays. Furthermore, while one potential use of AOPs is to apply data from the KERs to develop quantitative relationships, it also became clear that it is necessary to establish the appropriate kinds of data and to use only such data for an overall quantitative assessment of the relationship between the MIE and the AO. Overall, the lessons learned provide insight into the design of research and kind of data needed to optimally establish and use AOPs. Funded by ACC ARASP.

2654 A Cross-Study Analysis of Prenatal Exposures to Environmental Contaminants and the Epigenome: Support for Stress-Responsive Transcription Factor Occupancy as a Mediator of Gene-Specific CpG Methylation Patterning

E. Martin and R. C. Fry. *Gillings School of Global Public Health, Environmental Science and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

A biological mechanism by which exposure to environmental contaminants results in gene-specific CpG methylation patterning is currently unknown. We hypothesize that gene-specific CpG methylation is linked to environmentally-perturbed transcription factor occupancy. To test this hypothesis, we used *in silico* analysis to identify transcription factor binding sites enriched among genes with altered CpG methylation in cord blood leukocytes or placental tissue in relationship to a suite of environmental contaminants. A database of genes with altered CpG methylation either in cord blood leukocytes or placental tissue was compiled from 14 studies representing assessments of six environmental contaminants: arsenic, cadmium, manganese, mercury, lead, and tobacco smoke. For each study, the sequences of the promoter regions (-1000 and +500 bp from the transcription start site) for all genes with altered CpG methylation were analyzed *in silico* for enrichment of transcription factors binding sites. Across the 14 studies, a set of 396 unique genes was identified to have altered CpG methylation. Binding sites for a total of 56 unique transcription factors were identified to be enriched within the promoter regions of the genes. Binding sites for the Kidney-Enriched Krupple-like Factor 15, a known responder to endogenous stress were enriched ($p<0.001$-0.041) among the genes with altered CpG methylation associated with five of the six environmental contaminants. These data support the transcription factor occupancy theory as a potential mechanism underlying environmentally-induced gene-specific CpG methylation.

2655 Gene Expression in Breast Cancer Cells After Treatment with Mammary Carcinogens and Mammary Development Disruptors

J. Ackerman[2], R. Rudel[2], J. Yeakley[1], P. Shepard[1], A. Tagmount[3], A. Loguinov[3] and C. Vulpe[3]. [1]*Bio Spyder, Carlsbad, CA;* [2]*Silent Spring Institute, Newton, MA and* [3]*University of Florida, Gainesville, FL.*

Because of the challenges inherent in breast cancer epidemiology, *in vitro* and *in vivo* experimental evidence is crucial for identifying and understanding potential chemical causes of this disease. We have previously identified a set of 216 chemicals known to cause mammary tumors in rodents, and another 31 that disrupt mammary development. It is expected that mammary carcinogens exert their carcinogenic effects through diverse mechanisms, at the very least including estrogenic and genotoxic modes of action. We developed a panel of 313 genes related to processes involved in carcinogenesis, other hormone signaling, and other breast-cancer-related processes. We measured expression of this panel of genes in MCF 7 breast-cancer cells without treatment and after treatment with 5 agents: 17 beta estradiol (a classical estrogen), genistein (a phytoestrogen that affects breast development), perfluorooctanoic acid (a mammary carcinogen with non-estrogenic hormonal activity), tamoxifen (a selective estrogen response modulator with strong anti-estrogenic effects in mammary tissue), 1,4 benzoquinone (a genotoxic metabolite of the mammary carcinogen benzene), and vitamin C (a non-carcinogen with minimal endocrine or genotoxic activity). Exposures were conducted at three concentrations (1 nM-10 μM) and in the presence and absence of 10 nM estradiol. Gene expression was determined using the quantitative high-throughput RasL-seq platform. We cross-referenced these results with Gene Ontology annotations of differentially expressed genes. Notably, the genes differentially expressed in both estradiol- and genistein-treated cells (but not benzoquinone-treated cells) were enriched for many mammary-development-related terms. However, only the genes differentially expressed under estradiol treatment were enriched for mammary epithelial cell proliferation and differentiation. Thus, altered gene expression in a curated gene set related to breast development and cancer provides mechanistic insight into adverse outcome pathways relevant to breast cancer.

2656 User-Friendly Online Tools for ADMET Predictions Supporting the Risk Assessment of Cosmetics-Related Chemicals

A. N. Richarz[2], P. Alov[6], S. J. Enoch[2], S. Kovarich[3], T. Meinl[1], C. Mellor[2], A. Paini[5], A. Palczewska[4], J. V. Sala Benito[5], F. P. Steinmetz[2], M. T. Cronin[2] and A. P. Worth[5]. [1]*KNIME.com AG, Zurich, Switzerland;* [2]*Liverpool John Moores University, Liverpool, United Kingdom;* [3]*S-IN Soluzioni Informatiche SRL, Vicenza, Italy;* [4]*University of Bradford, Bradford, United Kingdom;* [5]*IHCP, European Commission Joint Research Centre, Ispra, Italy and* [6]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria.* Sponsor: C. Yang.

Computational methods support different aspects of chemical risk assessment, satisfying both regulatory requirements to reduce animal testing and the need to improve safety evaluation. Physiology-based kinetic (PBK) models that are combined with *in vitro* dynamics allow for *In Vitro* to *In Vivo* Extrapolation and prediction of concentration-time-profiles in the target organs considered, i.e. the internal exposure concentration. More than 20 PBK models have been developed within the EU COSMOS project, simulating relevant time profiles concentration for cosmetics related chemicals (e.g. caffeine, coumarin and their metabolites) for human, rat and *in vitro*, for different exposure (oral, dermal, inhalation) and target organs (blood, liver, lungs), as well as general models, not tailored for a specific chemical. The mathematical equations were programmed in R and the models then implemented into user-friendly workflows in the graphical KNIME user interface. They are supplemented by skin absorption and gastrointestinal absorption models facilitating oral to dermal extrapolation of data. To predict hazard and potential toxicity, *in silico* profilers were developed to screen target molecules for toxicophores, e.g. related to mitochondrial and liver toxicity or binding to nuclear receptors such as LXR/PPARy, which are associated with the adverse outcome pathway of liver steatosis. This suite of models addresses different steps in the safety assessment process and thus increases the weight of evidence in the evaluation. The novelty of these models lies in their transparency, flexibility and ease of access, users are able to adapt the workflows to their needs in the local desktop version, as well as their intuitive use through the COSMOS KNIME WebPortal (http://knimewebportal.cosmostox.eu), also including model documentation and user guidance. Supported by the EU FP7 COSMOS Project.

2657 Assessing Acute Toxicity by Combining Predictions Based on Chemotype Alerts, QSAR, and *In Vitro* and *In Vivo* Biological Assay Data in a Quantitative Weight-of-Evidence Approach

J. Rathman[1,3], T. Magdziarz[2], A. Mostrag-Szlichtyng[1], B. Bienfait[2], O. Sacher[2] and C. Yang[1,2]. [1]*Altamira LLC, Columbus, OH;* [2]*Molecular Networks GmbH, Erlangen, Germany and* [3]*Ohio State University, Columbus, OH.*

Acute oral toxicity testing on rats continues to be a regulatory requirement for many chemicals. There is a growing interest in developing computational methods for predicting acute toxicity in order to reduce animal testing and to enable more efficient compound prioritization and screening to, for example, determine the dose ranges for *in vivo* studies. We present a workflow for evaluating acute toxicity potential in which evidence from multiple sources is quantitatively combined to arrive at a weight-of-evidence prediction with associated estimation of uncertainty. *In silico* approaches, based on chemical structure and physicochemical properties are combined with biological data from both *in vitro* and *in vivo* studies. Predictive quantitative structure-activity relationship (QSAR) models of rat LD50 values were developed from structural and physicochemical descriptors and a number of chemotype alerts were also identified. While structure- and property-based *in silico* methods are suitable for modeling key molecular events, they are somewhat limited in their ability to adequately address subsequent biological processes. Experimental data from relevant biological assays were therefore employed in the workflow, including, for example, results from the *in vitro* neutral red uptake (NRU) cytotoxicity assay and *in vivo* zebrafish studies. The NRU assay, with normal human keratinocyte (NHK) and mouse embryonic fibroblast (BALB/c 3T3) cell lines, has been approved as an alternative method by EU ECVAM and US ICCVAM, and a portion of the results are available in the literature. *In vivo* assay results are available from the Tox21 and ToxCast programs, which together tested approximately 10,000 chemicals in the zebrafish assay. Unlike simple consensus strategies, the weight-of-evidence approach used here is a statistically rigorous method that takes into account the uncertainty associated with each individual evidence source. Including biological assay data in the prediction workflow enhanced the accuracy and reduced the uncertainty of acute toxicity predictions compared to predictions based on *in silico* methods (QSAR and chemotype alerts) alone.

2658 A Correlative Relationship Between Regional Clusters of Permethrin Exposure and Parkinson's Disease

B. Sie and D. E. Johnson. *Nutritional Sciences and Toxicology, UC Berkeley, Berkeley, CA.*

Parkinson's disease (PD) is a progressive neurological disorder that involves the loss of dopamine-producing neural cells of the substantia nigra. Only 10–30% of PD cases have a familial history, and previous investigation suggests a strong gene-environment component. One key gene related to PD is brain-derived neurotrophic factor (BDNF). BDNF has been widely used as a biomarker for the progression of PD. One key BDNF-interacting chemical is the pesticide permethrin. Permethrin represses membrane polarization in dopaminergic cells *in vitro*, which inhibits BDNF activation. Dermal absorption and inhalation exposure of permethrin are both sufficient to deliver permethrin through the rat blood-brain barrier and cause observable deficiency in sensorimotor performance. Several permethrin exposure assessments have been made; permethrin impregnated battle uniforms in Afghanistan and Germany, EPA SHEDS multimedia model in children, and CHMS and NHANES biomonitoring studies. Indoor dust samples from homes in Salinas Valley agricultural areas show permethrin as the highest contaminant. Therefore, it is plausible that long-term exposure to permethrin may increase the risk to PD susceptibility. In this investigation, an evaluation of this chemical-gene-disease linkage was made by comparing regional exposure to permethrin and regional prevalence of PD. Seventy total US counties with elevated and non-elevated exposure to agricultural permethrin were identified, and then compared by PD mortality rate. Counties with elevated production of high-permethrin crops such as corn exhibited PD mortality rates of 11.4 deaths per 100,000, with a 95% confidence interval (CI) of 10.2–12.5 deaths per 100,000. By contrast, control counties with similar population and life expectancy exhibited PD mortality rates of 6.3 deaths per 100,000 (95% CI 5.2–7.4). On average, counties with high agricultural permethrin exhibited PD mortality rates 79.6% higher ($p < 0.05$) than did control counties. Additionally, counties known for farming of high-permethrin crops exhibited a 116% higher ($p < 0.05$) occurrence of PD death than did counties known for farming similar volumes of non-permethrin crops such as oranges. This disease-pesticide association study suggests that permethrin exposure which may include commercial product exposure may add to the cumulative effects of chemically induced Parkinson's disease.

2659 Evaluation of *In Silico* Predictions of Acute Inhalation Toxicity for GHS Classification

M. DeLorme[2], C. Owens[2], J. Bonk[2] and L. Milchak[2]. [1]*Materials Resource Division, 3M, Saint Paul, MN and* [2]*Medical Department, 3M, Saint Paul, MN.*

One of the guiding principles of current toxicology research is the humane use of animals and the institution of the Three Rs, Reduce, Refine and Replace, especially in the area of acute toxicity testing. Various *in silico* models have recently been used to evaluate acute mammalian toxicity; However, the current ability to predict acute inhalation toxicity is very limited, particularly for chemicals with unique physical properties. The current study compared the GHS acute inhalation toxicity classification for a group of fluorochemicals based on the estimated rat inhalation LC50 from the commercially available TOPKAT QSAR software with the GHS classification based on historic rodent inhalation exposure data. The fluorocarbon materials were segregated into two phases: vapor and gas. For the vapor phase test materials TOPKAT predicted the animal based GHS classification for 42% of the test materials (n=43). For the incorrectly predicted vapor GHS classification, the prediction was more than two classification categories different than the animal based classification for 16 of the 25 incorrect predictions. Eight of the 25 incorrect categories over predicted acute toxicity (i.e. the model predicted a LC50 value that was lower than the actual LC50) while acute inhalation toxicity was under predicted for 17 chemicals (i.e. the model predicted a higher LC50 value than indicated by the actual LC50). For the gas phase test materials (n=8) the model did not correctly predict the correct GHS classification for any of the materials. However, unlike the vapor test materials, the order of magnitude of classification error was 2 categories or less. For 7 of the 8 test materials, the model over predicted acute inhalation toxicity and under predicted toxicity once. The lack of accuracy and precision may be readily attributable to the low number of fluorochemicals used to build the *in silico* LC50 model and also the general difficulty of predicting acute inhalation toxicity. The study emphasizes the need to validate *in silico* models prior to use, particularly for unique chemistries. Further development of *in silico* models for acute inhalation toxicity may need to incorporate not only chemical structure, but most likely other physical and chemical properties to increase prediction accuracy and precision.

2660 Using *In Vivo* Toxicity Syndromes to Reduce Uncertainty in Chemical Effects

R. Judson. *US EPA, Research Triangle Park, NC.*

In vivo toxicology data is subject to multiple sources of uncertainty: observer severity bias (a pathologist may record only more severe effects and ignore less severe ones); dose spacing issues (this can lead to missing data, e.g. if a severe effect has a less severe precursor, but both occur at the same tested dose); imperfect control of key independent variables (in databases, one can rarely control key input variables such as animal strain or dosing schedules); effect description heterogeneity (terminology changes over time which can lead to information loss); statistical issues (too few chemicals with a given phenotype, or too few animals in dose groups). These issues directly contribute to uncertainties in models built from the data. We are investigating the use of collections of endpoints (toxicity syndromes) to address these issues. These are identical in concept to medical syndromes which allow a physician to diagnose an underlying disease more accurately than can be done when relying on examination of one symptom at a time. Our test case is anemia, for several reasons: most of the phenotypes (e.g. cell counts) are quantitative; related effects are measured in an automated way; anemia is relatively common, at least at high doses (~30% of chemicals in our database show significant drops in red cell count); the causes of anemia are well understood; and, there is a standard clinical decision tree to classify anemia. Using a database of 658 chemicals, we have made several preliminary observations. Only a subset of chemicals show clear syndrome patterns that map to the standard clinical decision tree, which may reflect remaining noise in the data, or an indication that chemicals can lead to anemia through more than one route. Second, anemia often only occurs in a subset of species tested (e.g. rat, mouse, dog). This may be a reflection of the doses used, or a true cross-species effect. Finally, the majority of anemia occurs at relatively high doses, where significant weight loss and even mortality is seen. Overall though, the syndrome methodology appears to be a promising approach to classifying the toxicity of chemicals. This abstract does not necessarily reflect US EPA policy

2661 Evaluating the Proposition 65 Health Significance of Formaldehyde Exposures from Chinese Manufactured Laminate Flooring

P. Sheehan, K. T. Bogen, A. Singhal and R. Kalmes. *Exponent, Oakland, CA.*

Although formaldehyde emissions from Chinese manufactured laminate flooring recently have received national media attention, no published scientific studies address potential formaldehyde exposures to consumers who have installed such flooring. California's Safe Drinking Water and Toxic Enforcement Act of 1986, commonly referred to as Proposition 65, lists formaldehyde as a chemical known to the State to cause cancer and requires that products that emit formaldehyde either be shown to pose an exposure below the "safe harbor" daily dose for formaldehyde (40 µg/day) or be properly labeled as a carcinogen. We have conducted a Proposition 65 exposure assessment for several types of Chinese manufactured laminate flooring to assess consumer risk from flooring alone, as there are other formaldehyde sources in most homes. To estimate time weighted average (TWA) concentrations with time since installation, our evaluation used empirical formaldehyde emission rates derived from chamber testing of 100s of samples of installed laminate flooring and in-home samples of formaldehyde from sources including flooring in more than 1000 homes, along with a probabilistic model to address inter-household variability and associated uncertainties involving laminate coverage fraction and air-exchange rate. The model predicts that formaldehyde concentration attributable to the installed laminate flooring at the time of installation has an expected value of ~0.031 ppm (or 39 µg/m3). Mean duration of residence in the US is ~13 years, which according to our model is expected to lead to 83.3% decay in time-weighted average (TWA) formaldehyde emissions from laminate flooring over that period. Assuming a reference inhalation rate of 20 m3/day and 68% of each day spent at home, an average consumer is predicted to be exposed to an estimated lifetime TWA daily formaldehyde dose of <20 µg/day. A 1-tail 95% confidence bound of initial mean formaldehyde concentration based on estimation error is also expected to produce a lifetime TWA daily formaldehyde dose below the safe harbor level, clearly indicating that the Chinese laminate flooring evaluated in this assessment complies with the California's cancer-based safe harbor requirement.

2662 Toxicological Review of Hydrogen Sulfide Scavengers and Corrosion Inhibitors in Gas Pipelines

D. Johnson, Y. Yang and A. Pawlisz. *GHD, Dallas, TX.*

Specific chemical additives are introduced into pipelines designed to move extracted natural resources (e.g., oil and natural gas) from source to processing facilities. These chemicals include 2,2′,2″-(hexahydro-1,3,5-triazine-1,3,5-triyl)triethanol (HHTT; CASRN 4719-04-4), a scavenger for hydrogen sulfide (H2S), a naturally occurring chemical in oil and gas extraction, and 2,2′-thiobisethanol (TDG; CASRN 111-48-8), a corrosion inhibitor to reduce the corrosiveness of H2S. The accidental or operational (e.g., maintenance of valves) releases of these chemicals from pipelines may result in worker or other receptor exposure, thus a hazard assessment of these chemicals is warranted. Peer-reviewed publications, public databases, and subscription databases were searched for information related to physicochemical properties, environmental fate, and toxicological effects. Exposure assessment revealed that contact with HHTT is unlikely for workers because it reacts rapidly with H2S in the pipeline to form 2-(1,3,5-dithiazinan-5-yl) ethanol (DTE; CASRN 88891-55-8). Both DTE and TDG are highly water soluble, stable in the environment (low biodegradation), and may be mobile in soil. The high water solubility suggests that exposure to DTE and TDG may occur by incidental ingestion or inhalation of dust particles. While no laboratory toxicology data are available for DTE, a quantitative structure-activity relationship (QSAR) analysis was conducted using the OECD QSAR Toolbox to generate predictive toxicological values. Based on the QSAR analysis, the predicted no observed effect level (NOEL) for human exposure to DTE is 74.5 mg/kg-day. TDG causes irritation to the respiratory tract, skin, and eye, as well as gastrointestinal disturbance. TDG is rapidly metabolized and eliminated from the body. Toxicity to TDG only occurs at very high concentrations (i.e., >1,000 mg/kg). The no observed adverse effect level (NOAEL) for subchronic ingestion of TDG is 500-1,000 mg/kg in rats. These data suggest that there is limited risk related to worker (or other receptor) exposure to DTE and TDG from gas pipeline releases unless the exposure is at very high concentrations (i.e., >500 mg/kg-day), which is unlikely given the limited application rates of the scavenger/anticorrosive products.

2663 Levels, Sources, and Associated Risks for Inhalable Particulate Matter, Sulfur Dioxide and Polycyclic Aromatic Hydrocarbons in Curaçao

E. Pulster[1,3], G. Johnson[3], J. McCluskey[3], D. Hollander[3] and R. Harbison[3]. [1]*Mote Marine Laboratory, Sarasota, FL;* [2]*College of Marine Science, University of South Florida, Tampa, FL and* [3]*Environmental Occupational Health, University of South Florida, Tampa, FL.*

Complex refinery processes have been frequently associated with a wide variety of atmospheric emissions that can differ between refineries depending on the type of crude oil, operations, and refined products. One of the oldest and largest refineries in the Wider Caribbean region (WCR) is located on the island of Curaçao. The refinery, Isla Refineriá, has been the basis of historical environmental and human health debates and conflict between the public and the local government. This research aims to establish baseline levels of refinery associated atmospheric emissions [i.e., inhalable particulate matter (PM10), sulfur dioxide (SO2) and polycyclic aromatic hydrocarbons (PAHs)] and to evaluate potential public health risks in Curaçao. Available hourly, daily and monthly PM10 and SO2 measurements were downloaded from June 2010 through December 2014 from two local air monitoring stations. Passive air samplers with polyurethane foam collection disks (PAS-PUFs) were deployed in 2011 (n=43) and in 2014 (n=30) to measure ambient PAH concentrations. Concentrations of both PM10 and SO2 in Curaçao are among the highest reported globally, demonstrating an increasing trend over time and exceed current public health guidelines recommended by local and international agencies. Ambient PAH concentrations ranged from 1.2 ng/m3 in 2011 and 27.3 to 660.1 ng/m3 in 2014, demonstrating no temporal differences. However, there were highly significant spatial differences, with the samples downwind of the refinery having significantly higher ambient PAH concentrations than those upwind in 2014. Source elucidation revealed the ambient PAHs were dominated by petrogenic emission sources (i.e., refinery) in the 2011 and the 2014 downwind samples, whereas the 2014 upwind locations were equally influenced by both petrogenic (i.e., refinery) and pyrogenic (i.e., vehicle emissions) sources. Using the US EPA's risk analysis methodology the resulting cumulative lifetime cancer risk estimates from PAH inhalation were below the level of concern (1.0 x 10-4).

2664 Combined Incremental Lifetime Cancer Risk (ILCR) for Nitrosamines Present in Tobacco Smoke

S. A. Fiebelkorn, F. H. Cunningham and C. Meredith. *R&D Centre, British American Tobacco, Southampton, United Kingdom.*

Tobacco smoke contains over 6,000 constituents, at least 150 of which have established toxicological properties. Eight nitrosamines have been identified by the US FDA as Harmful and Potentially Harmful Constituents (HPHC) in tobacco smoke, 4-N-Nitrosomethylamino-1-(3-pyridyl)-1-butanone (NNK), N-Nitrosonornicotine (NNN), N-Nitrosodiethylamine (NDEA), N-Nitrosodiethanolamine (NDELA), N-Nitrosodimethylamine (NDMA), N-Nitrosoethyl-methylamine (NEMA), N-Nitrosopiperidine (NPIP) and N-Nitrosopyrrolidine (NPYR). Incremental Lifetime Cancer Risk (ILCR), can be used to prioritise toxicants based on published cancer potency values and estimated human exposure from smoking. Inhalation unit risks (IUR) were available for five of the nitrosamines (NDEA, NDMA, NEMA, NPIP and NPYR) all of which were derived from the oral cancer slope factor (CSF) by either the US EPA or OEHHA. For a further three nitrosamines (NNK, NNN and NDELA) only the oral CSF were available (US EPA or OEHHA) and these were converted to risk per µg/m3 assuming a default human daily breathing volume of 20m3 and 70kg body weight. IUR values for the nitrosamines were all based on either rat or hamster data, for liver, respiratory or combined tumour incidence. The ILCR for each nitrosamine was calculated by multiplying the IUR by an estimated daily exposure to tobacco smoke based on machine smoked yields using the Health Canada Intense (HCI) smoking regime from a 3R4F reference cigarette. The yields used are either based on actual measured values, or limit of detection (LOD) values and were as follows: NNK 0.252 µg/cig; NNN 0.282 µg/cig; NDEA 0.000617 µg/cig (LOD); NDELA 0.000758 µg/cig; NDMA 0.00271 µg/cig; NEMA 0.000509 µg/cig (LOD); NPIP 0.000172 µg/cig (LOD); NPYR 0.000396 µg/cig (LOD) The ILCRs were then ranked from highest to lowest as follows: NNK 3.53E-3; NNN 1.13E-4; NDEA 2.65E-5; NDELA 6.06E-7; NDMA 3.79E-5; NEMA 3.21E-6; NPIP 4.64E-7; NPYR 2.42E-7For the nitrosamines, the combined increased lifetime cancer risk due to continuous smoking exposure, based on a simple additivity model, was calculated as 0.00371. NNK was estimated to contribute to approximately 95% of this risk. All the data used for this assessment is based on oral exposure, not inhalation data, and the majority of data sets are for liver tumours and not lung lesions. Further assessment based on inhalation data would be recommended as and when data becomes available.

2665 Comparisons of Isotope Dilution Method/Solid Phase Microextraction and Laboratory Models to Estimate Risk of DDTs from Consumption of Fish from Contaminated Sediments in Palos Verdes, California

S. L. Coffin, G.-b. Xu, J. Gan and D. Schlenk. *Environmental Toxicology, University of California, Riverside, Riverside, CA.*

DDT and its primary metabolites are a major source of concern in the Palos Verdes Shelf. Biota-sediment accumulation (BSAF) and biomagnification (BMF) are known factors that cause increased DDT exposure through fish consumption. In this study, BSAF and BMF values were modeled chemically from bioavailability derived using one of two methods, including isotope dilution methods (IDM) and solid phase microextraction (SPME) which were then coupled with a biological laboratory experiment consisting of the benthic polychaete worm, *Neanthes arenaceodentata* and the demersal flatfish, *Pleuronichthys verticalis* as a model species for benthic fish that are targeted for human consumption such as California halibut (*Paralichthys californicus*). Using human seafood-consumption data specific to anglers in the Palos Verdes area, cancer and non-cancer risks were calculated and compared to field data assessments. Comparisons were made for the general public as well as high-risk groups. The following values represented exposure to California halibut consumption only. The calculated BMF and BSAF values in this study (4.09 ±0.82 and 3.0x10-3 ± 9.1x10-4, respectively) were comparable to previous values determined from other regulatory agencies (4.06 (n/a) and 2.6x10-3 ± 9.3x10-4, respectively). Cancer risk to the general public (50th percentile) and an at-risk group (private boat anglers, 95th percentile) were also determined (1.21x10-8 ±6.27x10-11 and 3.49x10-7 ± 7.78x10-10) and were also similar to previous values (2.48x10-8 ±8.9x10-9 and 3.08x10-7 ±1.1x10-7, respectively). The calculated non-cancer hazard quotient values for general public and private boat anglers from this study (4.14x10-4 ± 2.15x10-6 and 5.13x10-3 ±2.67x10-5, respectively) were also within the same order of magnitude of earlier studies (8.51x10-4 ±3.04x10-4 and 1.06x10-2±3.78x10-3, respectively). Given the similarities of the risk value estimates determined by IDM and field data, these laboratory models may be used to accurately predict bioavailability (NIEHS/SRP R01ES020921;R01ES024313).

2666 Risk Assessment of Even-Carbon-Chain Alkanes (C12-C28) as Extractables from Bioprocessing Bags

K. L. Li and D. Dolan. *Environment, Health, Safety and Sustainability, Amgen Inc., Thousand Oaks, CA.*

In biomanufacturing, disposable bioprocessing bags are often used for buffer preparation. Such applications may require anti-static bags to mitigate the physical hazards associated with static charges. As part of the qualification testing, we studied the extractables profile of an anti-static bag (Supplier X, Film Y) under experimental conditions (40° C for 4 days). A total of 18 organic compounds were reported in ethanol extracts (range: 0.2 to 10 mg/bag) by GC/MS, HPLC/UV and HPLC/MS. The profile consisted of transformed anti-oxidants, nylon fragments, slip agents and n-alkanes. Based on the point of application of the anti-static bag, stepwise clearance of the in-process extractables was estimated. After normalizing the levels by surface area and extraction volume, and taking clearance into account, the theoretical residual extractables levels in final drug products were toxicologically insignificant. The n-alkanes were unique in that the cluster was all even-carbon-chain-alkanes (C12-C28). Available toxicology information supplemented with QSAR was compiled on the individual alkanes. In summary, eicosane (C20) had the most extensive and robust dataset. None of the alkanes showed potential for *in vitro* mutagenic activity. A few of the compounds had direct or indirect food additive use (C12, C14 and C20). A few were reported to occur naturally in foods (C12-C16). No toxic metabolites were noted. Overall, the C12-C28 alkanes were shown to have a low order of systemic toxicity by the oral and parenteral routes. Based on a developmental and teratogenicity study in rats, the maternal and fetal NOAEL values for eicosane (C20) was 1000 mg/kg, the highest dose tested (ECHA 2014). Based on the principles and methods of the ICH Q3C(R5), a parenteral Permitted Daily Exposure (PDE) value of 120 mg/day was derived for the even-carbon-chain alkanes (C12-C28) using eicosane as the indicator. Using this approach, the theoretical estimates of the residual alkanes in the final drug products were orders of magnitude below the parenteral PDE.

2667 Assessment of Dietary Exposures to Inorganic Arsenic from Rice and Compliance with California Proposition 65

L. A. Haighton, K. Ly, H. Fikree, T. Jonaitis and J. W. Card. *Intertek Scientific & Regulatory Consultancy, Mississauga, ON, Canada.*

Inorganic arsenic or "iAs" may occur in soils from natural sources or from past uses of arsenical herbicides, etc. Rice consumption is a top source of dietary exposure to iAs as rice crops readily absorb iAs from soils. California Proposition 65 requires businesses to provide a warning if a product contains a carcinogen (e.g., iAs) at levels that would result in exposures above a Safe Harbor Level (SHL). The SHL for (oral) iAs is 10 µg/day. OEHHA recently proposed that 0.06 ppm (white) and 0.130 ppm (brown) of iAs in unprocessed rice can be considered as naturally occurring for determining warning requirements. In deriving these values, analytical data on white and brown rice samples from California were considered, with the proposed natural estimates corresponding to the mean iAs value of the samples. Median values were slightly higher, suggesting that more than half of rice sold in California could potentially require a warning or become the target of bounty hunters. The highest amounts detected in the rice samples were 0.10 ppm (white) and 0.20 ppm (brown). To determine if the SHL would likely be exceeded, we used NHANES 2011–2012 data to calculate the arithmetic means of white and brown rice consumption among users in the US: results were 109 g/day (white) and 99 g/day (brown). Using the results from NHANES, total exposures to iAs if present at 0.10 ppm in white rice would be 10.9 µg/day, and if present at 0.20 ppm in brown rice would be 19.8 µg/day, which exceed the SHL. If "natural" amounts are subtracted, as recommended in the proposed regulation [0.10 - 0.06 ppm = 0.04 ppm (white); 0.20 - 0.13 ppm = 0.07 ppm (brown)], exposures to iAs present in rice due to non-natural sources would be below the SHL [4.36 µg/day (white) and 6.93 µg/day (brown)], suggesting that dietary exposure to iAs in rice is at levels that would not require a Proposition 65 warning. Note that if concentrations are likely to exceed those considered above, an alternative could be to establish a standard warning for iAs in rice as is commonly utilized with mercury in fish.

2668 Application of a Weight of Evidence Approach to Evaluating Risks Associated with Subsistence Caribou Consumption Near a Lead/Zinc Mine

M. R. Garry[1], S. S. Shock[1] and J. H. Salatas[2]. [1]*Exponent, Bellevue, WA and* [2]*Teck Cominco, Anchorage, AK.*

Caribou are an integral part of life for native northern Alaskans for both subsistence and cultural reasons. The Red Dog Mine area is highly mineralized and historical overland transport of ore concentrate from the Red Dog lead/zinc mine in northwest Alaska to the seaport raised concerns regarding subsistence use of caribou. Sampling results indicated the presence of lead in road soil and on moss growing near the road. Caribou herds range widely, but sometimes overwinter near the road. We applied a weight of evidence approach, using multiple lines of evidence, to evaluate potential risks associated with subsistence consumption of caribou harvested near the road and mine. The components included 1) a human health risk assessment using standard EPA risk paradigm with site-specific assumptions, 2) a long-term caribou monitoring program to evaluate trends in metals (lead, cadmium, and zinc) concentrations in caribou (muscle, liver, and kidney tissue), 3) comparison of metals concentrations in locally harvested caribou relative to other sources of meat, and 4) a site use study based on analysis of data collected from satellite-collared caribou from the Western Arctic Herd (WAH). The risk assessment predicted risks below acceptable limits for exposure to metals from subsistence caribou consumption (HI≤0.3; <0.0005% chance blood lead ≥10 µg/dL). Monitoring program data indicate a lack of consistent trends for either increasing or decreasing metals loads. Lead, cadmium, and zinc from all tissues were within the range of reference concentrations reported for caribou elsewhere in Northern Alaska. Finally, the site use study showed that caribou utilize the area near the road, port, and mine approximately 1/20th to 1/90th of the time assumed in the risk assessment, implying that risks were significantly overestimated in the risk assessment. The results from multiple lines of evidence consistently indicate that fugitive dust emissions from Red Dog Operations do not appear to be a significant source of metals in caribou, and that caribou remain safe for continued subsistence harvesting.

2669 Flame-Retardants in Upholstered Furnishings: An Assessment of Health Risk and Fire-Related Deaths in the Era of California Technical Bulletin (TB-117)

L. Gloekler[1], E. Shay Hynds[2], N. Schmidt[1], N. Haghighat[1], J. Panko[2], D. Cowan[1] and D. Paustenbach[3]. [1]*Cardno ChemRisk, Aliso Viejo, CA;* [2]*Cardno ChemRisk, Pittsburgh, PA and* [3]*Cardno ChemRisk, San Francisco, CA.*

California's upholstered furniture flammability standard, Technical Bulletin 117 (TB-117), was first established in 1975 and updated in 2013. Furniture manufacturers added flame retardant chemicals to polyurethane foam to comply with TB-117, which originally required filling materials to meet an open flame test. In the 2013 update, focus shifted to cover fabrics and smoldering as the primary ignition source, thereby allowing the standard to be met without the use of flame retardants. Concern has grown for the adverse human health effects of flame retardants and whether these health effects outweigh the potential benefits. The purpose of this study was to 1) conduct a human health risk assessment of flame retardants currently found in polyurethane foam and 2) evaluate fire incidence and associated injuries and deaths in California. All non-cancer hazard indices calculated were well below the US EPA HI benchmark of 1, and, for one applicable chemical, the cancer risk was well within the US EPA's acceptable risk range. Total fire injuries and deaths, including fires in which upholstered furniture was the first item ignited, decreased significantly in California residential buildings between 1980 and 2014. The decrease in fire-related deaths in California was more pronounced in comparison to Florida, a state without stringent fire regulations. Our analysis showed that there are no health risks concerning flame retardants for which both toxicity criteria and concentration data were available, and there are potential quantifiable benefits as demonstrated through the decline in fire injuries and deaths. The lack of publically available flame retardant manufacturing data and the uncertain impact of confounding factors (e.g. smoking prevalence and smoke alarms) make it difficult to conclude or quantify with certainty the impact flame retardants in furniture has had on the decline of fire injuries and deaths.

2670 Risk Assessment of Chemical Exposures from Artificial Turf

M. K. Peterson[1], S. Pacheco Shubin[2] and T. A. Lewandowski[2]. [1]*Gradient, Leavenworth, WA and* [2]*Gradient, Seattle, WA.*

Artificial turf athletic fields have been growing in popularity over the past several decades due to decreased maintenance, water, and energy costs relative to conventional fields. However, since elevated levels of lead were discovered in a few turf products in the early 2000s, concerns have been raised repeatedly about potential chemical exposures associated with use of this product. Using a variety of search techniques, we compiled all the currently available data on chemical exposures associated with artificial turf fields and prepared a risk assessment that evaluated dermal, ingestion, and inhalation exposures. In general, exposures were well below acceptable risk assessment limits, with the exception of those for a few chemicals, for which concern was limited because bioavailability is low. In the screening analysis, 51/55 (93%) of the chemicals were detected at levels below those specified in risk assessment guidelines for soil, and 60/60 (100%) were below limits for leachate (indicating a low concern for groundwater contamination). Although only limited data were available for a few turf components (e.g., carbon black, nanomaterials), resulting in some uncertainty, our analysis found that users of artificial turf are unlikely to face increased risks of health effects, and that exposures are similar to those that might be found from playing on normal urban soil. Our findings are consistent with those of earlier studies by multiple regulatory agencies, which also suggested a low risk.

2671 Risk from Breaches in Portable, Consumer Sized Lithium Batteries

C. Y. Chan, M. Krause, B. Kelman and N. Moore. *Veritox Inc, Redmond, WA.*

Although lithium (Li) batteries in portable consumer electronics generally do not pose a hazard when used as intended in normal environments, "thermal run-away" reactions occur upon abusive treatment of batteries (such as elevated temperature storage, physical abuse induced short circuiting, or overcharging). Thermal run-away reactions lead to battery venting and release of smoke, which correspond with consumer reports of Li batteries "discharging" from personal electronics. We assessed whether inhalation exposure to hydrogen fluoride (HF), a decomposition reaction product of Li batteries, posed health hazards to

nearby consumers. We performed literature search for measurements of emissions from Li batteries under conditions of thermal runaway and combustion. We then conducted a safety assessment of exposure to HF during Li battery breaches resulting from (1) thermal run-away or (2) fire engulfment. Although burn injuries and smoke inhalation are of most significant concern in a fire, there is a general question of whether inhalation of released HF gas is also a hazard. We compared our Near Field-Far Field (NF-FF) modeled "worst case" scenario exposure levels for vaporization/aerosolization of HF released for each condition in a 10′ by 10′ by 8′ room with the Acute Exposure Guideline Levels (AEGLs). We found no increase in risk from HF with thermal run-away conditions. In contrast, based on laboratory fire testing, levels of HF released during a battery fire could be hazardous, depending on the battery's state of charge (SOC) when burned. For example, when we modeled NF-FF emissions from a burning AA-sized battery, maximum estimated HF concentrations were largest for a dead battery (0% SOC) with peaks of 37 mg/m^3 near the burning battery and 31 mg/m^3 within the room. Assuming a nominal air changes per hour (ACH) of 0.44, these levels correlated with 1-hr TWAs of 25 to 26 mg/m^3, which were >30 times the AEGL-1 level (0.8 mg/m^3), 1.3 times the AEGL-2 (disabling) levels (20 mg/m^3), but below the AEGL-3 (lethal) levels (36 mg/m^3). In comparison, burning fully charged (100% SOC) or partially charged (50% SOC) batteries resulted in exceeding AEGL-1 levels but not AEGL-2 or AEGL-3 levels. Thus, our assessment indicated that exposure to HF released from a Li battery during thermal run-away do not pose a risk, but could be a hazard if the battery is engulfed in fire.

PS 2672 Potential Tremolite Exposures Associated with Talc-containing Products

A. M. Burns and B. L. Finley. *ChemRisk, Cardno, Brooklyn, NY.*

Talc is a mineral that has been used in a number of applications such as talcum powder, rubber, paper, paints, pharmaceuticals and drywall accessory products. The two general grades of commercial talc, industrial and cosmetic, are sold depending on functional or physical characteristics required for specific applications (IARC 1987). Talc has been mined in over 40 countries including the United States (IARC 1987). The composition of talc can vary widely from one geological deposit to another where various mineral ores and silicates such as tremolite, anthophylite, and serpentines may be present (IARC 1987). Talc is primarily in the form of plates, however, rarely can be fibrous in nature (ACGIH 2001). Some concerns have been raised regarding the potential for human exposure to fibrous tremolite in talc-containing consumer products. We previously established a No Adverse Effect Level (NOAEL) for fibrous tremolite to be between 0.05 and 2.6 f/cc-year (Finley et al. 2012). The purpose of this assessment was to understand the potential direct and bystander exposures to fibrous tremolite during residential and occupational use of talc-containing drywall accessory products, assuming tremolite is present and in its fibrous form. Using personal airborne asbestos exposure measurements, and published frequency and duration data for the residential and occupational scenarios, cumulative fibrous tremolite exposures were estimated. The cumulative exposure for direct work ranged from 0.000071 to 0.41 f/cc-year for the residential and occupational scenarios, respectively. The bystander exposures ranged from 0.000017 to 0.098 f/cc-year for the residential and occupational scenarios, respectively. In all cases, the cumulative exposure estimates were well below or within the range of the published NOAEL for fibrous tremolite, which indicates that the presence of talc in joint compound does not pose an asbestos risk. This is consistent with the lack of asbestos-related diseases reported for drywall workers.

PS 2673 A Hazard and Risk Assessment of Phenoxyethanol in Children's Personal Care Products

K. Zu, J. Lemay, J. Zhang and J. Goodman. *Gradient, Cambridge, MA.*

Phenoxyethanol is a common preservative used in cosmetic products to prevent bacterial growth. Research and regulatory agencies worldwide conclude that phenoxyethanol poses no risk if it makes up less than 1% of a cosmetic product. Recently, concern has arisen that the regulatory limit may not provide an adequate margin of safety for infants and small children. Therefore, we conducted a hazard and risk assessment for phenoxyethanol in children's personal care products. We first conducted a hazard assessment of phenoxyethanol in its neat form, considering both dermal and oral exposure. We found that, although no studies have directly evaluated the carcinogenicity of phenoxyethanol, available evidence indicates a lack of genotoxicity. Also, laboratory animals dermally exposed generally experienced no adverse effects. Following oral exposure, some toxicity occurred at very high doses. In two-generation studies, no adverse effects were observed in offspring unless the dose was high enough to cause maternal toxicity. We identified the points of departure (PODs) of phenoxyethanol from the hazard assessment, then calculated children's exposures from using multiple personal care products containing phenoxyethanol. We evaluated one common brand and divided its products for children into two broad categories: lotion/creams and bathing products. For each category, we selected the products with the highest concentrations of phenoxyethanol to calculate hypothetical exposures. We estimated both typical and high-end exposures possible for a child using regular lotions, sunscreen lotions during time outdoors, and body wash at bath time. Last, we performed a margin of exposure (MOE) analysis, comparing exposure scenarios to the PODs identified in the hazard assessment. For the typical exposure scenario, the MOE is 1,661 and 208 for oral and dermal exposure, respectively. For the high-end exposure scenario, the MOE is 239 and 20 for oral and dermal exposure, respectively. We conclude that the available evidence indicates that phenoxyethanol, present at <1% of formula in personal care products, does not pose a health risk to infants or small children.

PS 2674 Integrating Toxicokinetic Data into Weight of Evidence Analysis Supports a Threshold-Based Risk Assessment for 1,3-Dichloropropene via Inhalation Exposure

Z. J. Yan[1], S. C. Gehen[1], M. M. Bartels[2], J. J. Hotchkiss[2] and R. J. Rasoulpour[1]. [1]*Dow AgroSciences, Indianapolis, IN and* [2]*The Dow Chemical Company, Midland, MI.*

Inhalation exposure to high concentrations of 1,3-Dichloropropene (1,3-D), a soil fumigant, has been shown to result in increased benign brochioalveolar adenomas in male B6C3F1 mice at 60 ppm, but is non-tumorigenic in female B6C3F1 mice and either sex of F344 rats at this same exposure concentration. In order to understand the potential relevance of this increase in benign lung tumors in male mice to human health risk assessment, a toxicokinetic study was performed to determine if there was saturation of metabolic processes at or near 60 ppm. In this study, male B6C3F1 received 6-hour exposures to 2.5, 5, 10, 20, 60 or 150 ppm 1,3-D. Following exposure, blood kinetics of 1,3-D was measured along with minute volume respiration rates. Analysis of these data revealed that blood concentrations of 1,3-D exhibited clear nonlinearity at and above 60 ppm, due to a change in absorption/metabolism and a depression in respiration rate and inhaled volume of test material at high exposure concentrations. These data demonstrate that the pulmonary tumorigenicity of 1,3-D occurred only at exposure levels exceeding a kinetically-derived maximum dose (KMD). 1,3-D is non-genotoxic in intact animals, as supported by negative results from all guideline- and GLP-compliant *in vivo* studies including mouse bone marrow micronucleus test, dominant lethal assay, Big Blue mouse mutagenicity and 32P post-labeling studies. Particularly, Big Blue mouse and 32P post-labeling studies both assessed potential genotoxicity in the lung above the concentration at which increased tumors were observed. Further, across all repeat-dose and carcinogenicity studies, a clear point of departure (POD) of 5 ppm, the same as the chronic NOAEL, was identified at which no systemic effects or tumors were induced. The chronic inhalation risk estimates in the high-use areas were well above the level of concern as recognized by US EPA (2008). Overall, no tumorigenicity at concentrations below KMD, negative *in vivo* genotoxicity and a clear no effect POD protective of development of lung tumors that occurs above 60 ppm support a threshold-based risk assessment for 1,3-D.

PS 2675 Perfluorinated Compounds at California Hazardous Waste Sites

K. C. Gettmann, L. Nakayama Wong and M. J. Wade. *Dept Toxic Substances Control, Cal/US EPA, Sacramento, CA.*

Perfluorinated compounds (PFCs) are man-made, fully fluorinated, organic compounds that persist in the environment, resist environmental degradation, and bioaccumulate. PFCs are an "emerging contaminant," and a threat to human health and the environment. They can be found in all media (groundwater, surface water, sediment, soil, and air). Long-chain PFCs such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are of primary concern; however, there are other short-chain PFCs. PFOS and PFOA are high production volume chemicals used in consumer and industrial products like stain removers and aqueous film forming foam (AFFF). Recently PFCs have been detected at California hazardous waste sites at former firefighting training areas, where sampling was conducted in soil, sediment, and groundwater for PFCs. At Site A, PFOS was detected in influent and effluent samples from a groundwater treatment system at 0.235 µg/L and 0.233 µg/L, respectively. At Site B, PFOS was detected in groundwater at 0.064 ug/L and PFOA was detected at 0.27 µg/L. We developed tapwater DTSC-target levels using DTSC recommended exposure parameters and provisional

toxicity criteria for PFOS (0.57 µg/L) and PFOA (0.4 µg/L). At Site A, the PFOS noncancer hazard quotient (HQ) is 0.4 for both groundwater detections and at Site B the HQ for PFOS is 0.1 and 0.7 for PFOA. There are limited toxicity criteria and screening levels (SLs) for PFCs. USEPA established drinking water short-term exposure provisional health advisory (PHA) levels of 0.2 µg/L for PFOS and 0.4 µg/L for PFOA. Given PFCs functional use as a water-, oil-, and stain repellent, DTSC's Safer Consumer Products (SCP) Priority Product Work Plan currently lists them as potential candidate chemicals in products related to buildings, furniture and furnishings, and clothing. The lack of toxicity criteria and limited SLs available for PFCs make conducting human health and ecological risk assessments challenging at hazardous waste cleanup sites and when evaluating consumer products.

PS 2676 Ion Channel Studies Support the Safety of Triclosan to Cardiovascular and Skeletal Muscle

D. Urbach-Ross, B. Slezak and D. Bagley. *Global Product Safety, Colgate-Palmolive, Piscataway, NJ.*

Triclosan (TCS) is an antimicrobial compound found in a wide range of consumer products. It was previously reported that TCS impaired cardiac and skeletal muscle function in mice and *in vitro*. The aim of these studies was to determine the impact of TCS, if any, on cardiovascular and skeletal muscle at a range of concentrations including those measured in human plasma following exaggerated brushing with a TCS containing toothpaste. To study the cardiac ion channel-blocking potential of TCS, an ion channel panel was conducted, measuring current recorded from the hERG channel in a stably transfected human embryonic kidney (HEK293) cell line and cardiac sodium (INa) and calcium (ICa) channels from isolated human atrial myocytes. Test concentrations of 0.001, 0.01, 0.1, 1.0, and 10 µM (n = 5) were examined. Since >95% of TCS is bound to plasma protein in humans, this study was done in the presence and absence of human serum albumin (HSA). In the presence of HSA, TCS was associated with no significant changes in the parameters measured, including hERG, INa, and ICa current. In the absence of HSA, TCS did not affect hERG and INa current and only showed a reduction of ICa current amplitude at the two highest concentrations. To study the potential for effects on skeletal muscle, a separate study examined ICa recorded from skeletal myocytes at 0.001, 0.01, 0.1, 1.0, and 10 µM (n = 5) in the absence and presence of HSA. In the absence of HSA, only the two highest doses reduced ICa current amplitude. In the presence of HSA, a reduction in current amplitude was only observed at the highest dose tested. The no observable effect concentration (NOEC) from these studies (0.1 µM w/out HSA and 1.0 µM w/HSA) is more than 100-fold higher than plasma levels measured following exaggerated brushing with a TCS containing toothpaste. Therefore, these results support that physiologically relevant doses of TCS would not be expected to have adverse effects on cardiovascular and skeletal muscle.

PS 2677 Integrating the Threshold of Toxicological Concern (TTC) with High Throughput Exposure Assessment for Risk-Based Screening of Several Thousand Commodity Chemicals

R. Becker[1], J. Wambaugh[4], G. Patlewicz[4], S. Felter[2] and T. Simon[3]. *[1]American Chemistry Council, Washington, DC; [2]Procter & Gamble Company, Mason, OH; [3]Ted Simon LLC, Winston, GA and [4]NCCT, US EPA, Research Triangle Park, NC.*

Although progress has been made with HTS (high throughput screening) in profiling biological activity (e.g., EPA's ToxCast), challenges arise interpreting HTS results in the context of adversity & converting HTS assay concentrations to equivalent human doses for the broad domain of commodity chemicals. Here, we propose using TTC as a risk screening method to evaluate exposure ranges derived from NHANES for 7968 chemicals. Because the well-established TTC approach uses hazard values derived from *in vivo* toxicity data, relevance to adverse effects is robust. We compared the conservative TTC (non-cancer) value of 90 ug/day (1.5ug/kg/day) (Kroes et al., Fd Chem Toxicol, 2004) to quantitative exposure predictions of the upper 95% credible interval (UCI) of median daily exposures for 7968 chemicals in 10 different demographic groups (Wambaugh et al., Environ Sci Technol. 48:12760-7, 2014). Results indicate: (1) none of the median values of credible interval of exposure for any chemical in any demographic group was above the TTC; & (2) fewer than 5% of chemicals had an UCI that exceeded the TTC for any group. However, these median exposure predictions do not cover highly exposed (e.g., occupational) populations. Additionally, we propose an expanded risk-based screening workflow that comprises a TTC decision tree that includes screening compounds for structural alerts for DNA reactivity, OPs & carbamates as well as a comparison with bioactivity-based margins of exposure (Wetmore et al., Toxicol. Sci., 2015). This TTC risk-based screening approach may be useful in a modernized TSCA as the first step in risk-based prioritization. Subsequent steps for substances not deprioritized by this TTC method could include analysis using HTS bioactivity/mechanistic screening & read-across as part of integrated testing or exposure refinement. This abstract does not necessarily reflect US EPA policy.

PS 2678 Inhalation TTC Values for Compounds Released into the Breathing Circuit of Respiratory Devices

R. Brown, A. Deng, C. Cruse and K. Riaz Ahmed. *US FDA, Silver Spring, MD.*

Patients can be exposed to volatile organic compounds (VOCs) by inhalation following their release into the breathing circuit of respiratory medical devices (e.g., ventilators). The risk posed by patient exposure to these compounds can be assessed by comparing a Tolerable Intake (TI) value for the VOC to the dose of the compound received during use of the device; however, toxicity data are not available to derive TI values for a number of VOCs released from device materials. In this case, an inhalation Threshold of Toxicological Concern (TTC) value can serve as a default TI value in the absence of toxicity data needed to derive compound-specific TI values. To develop the proposed inhalation TTC values applicable for compounds released by polymeric materials in the gas pathway of respiratory devices, a data mining exercise was conducted to identify and analyze relevant inhalation toxicity data in the RepDose database. To ensure that the applicability domain of compounds in the database was representative of compounds likely to be released from device-related polymers, pesticides and other highly potent compounds were eliminated from the analysis. Two-dimensional visualization and comparison of the chemical space of selected compounds from the RepDose database to VOCs known to be released from respiratory devices indicated that the compounds in each data set were structurally similar. The 10th percentile of the distribution of NOAEL values of compounds in the RepDose with subacute (≤ 30 days) toxicity data served as the basis for the proposed short-term inhalation TTC values for children and adults of 20 and 300 µg/day, respectively. Proposed longer-term (> 30 days) inhalation TTC values for children and adults of 1 and 10 µg/day, respectively, were derived from subchronic and chronic duration studies in the database. Use of these proposed inhalation TTC values will allow regulatory agencies and medical device manufacturers to assess the risk posed by patient exposure to VOCs released into the gas pathway of respiratory devices in the absence of inhalation toxicity data for these compounds.

PS 2679 Single vs. Multiple Target Organ Identification in Target-Organ-Specific Hazard Index (TOSHI) Chemical Mixture Risk Assessment

D. M. Pizzurro and G. I. Greenberg. *Gradient, Cambridge, MA.*

US EPA guidance for evaluating non-cancer hazards in risk assessment of chemical mixtures recommends identifying substance-specific target organs to calculate a target-organ-specific hazard index (TOSHI). Because of the general lack of mode of action (MoA) and pharmacokinetic information, target organ identification (TOI) is often used to approximate toxicological similarity among different substances. Although no explicit protocol for TOI exists, the target organ for a particular chemical can be identified from the critical effect in the principal study chosen as the basis for the corresponding toxicity reference value used in the risk assessment underway (e.g., the basis for an EPA IRIS value). Designating a single target organ for chemicals when multiple target organs are indicated in this basis for the reference value (e.g., trichloroethylene, ethylbenzene) may add substantial uncertainty to the risk assessment and ultimately underestimate risk. Our analysis of hazard indices (HIs) for a select group of chemicals in a simulated chemical mixture risk assessment compared traditional summation HI values, TOSHI values for a single target organ, and TOSHI values for multiple target organs. Our analysis revealed that, for these chemicals, multiple target organ TOSHI values are more conservative (i.e., closer to a hazard quotient of 1) than single organ TOSHIs, but slightly lower than HI summation values across all substances with no consideration of toxicological similarity. Although all three methods contain assumptions and uncertainties, we conclude that, for the selected chemicals, multiple target organ TOSHI analysis provides a sufficiently health-protective value that also considers substances' potential to preferentially affect more than one target organ. We discuss other key considerations and uncertainties that affect TOSHI derivation in chemical mixture risk assessment, including TOI for systemic/generic effects (e.g., decreased bodyweight), using developmental or reproductive categories as their own "target organs," and high dose effects as they relate (or not) to a substance's MoA at plausible exposure levels.

2680 Derivation of Data Derived Extrapolation Factors for the HPPD Inhibitor Mesotrione for Human Health Risk Assessment

E. Scollon[1] and J. Botham[2]. [1]*Syngenta Crop Protection, LLC, Greensboro, NC and* [2]*Syngenta Limited, Bracknell, United Kingdom.*

The US EPA human health risk assessment incorporates several default uncertainty factors (UFs), including an interspecies (10x), intraspecies (10x), and a FQPA factor when necessary. The 10x factors are subdivided into 3x for pharmacodynamics and 3x for pharmacokinetics. Mesotrione is a triketone herbicide that inhibits 4-hydroxyphenylpyruvate dehydrogenase (HPPD). HPPD is part of the tyrosine catabolic pathway in plants and animals; inhibition results in plant bleaching and accumulation of tyrosine in animal tissues. The EPA risk assessment uses the opaque and cloudy eyes from F1 mice from the reproduction study as the key effect to derive the point of departure in order to estimate risk. The effect is attributed to excess plasma tyrosine. The present work uses a data-derived extrapolation factor (DDEF) rather than default UFs for the human health risk assessment. The proposed DDEF is based on similarity of response of the rate-limiting tyrosine aminotransferase (TAT) enzyme between mice and humans. TAT converts tyrosine into the intermediate 4-hydroxyphenylpyruvic acid (HPPA) and determines the tyrosine plasma concentration in mammals. TAT activity for mice and humans are similar, 7.8, 10.5, and 7.17 nmol/HPPA/min/mg in male and female mice and humans, respectively. When HPPD is completely inhibited, peak plasma tyrosine concentrations are similar between mice and humans. Therefore, the pharmacokinetic portion of the interspecies UF can be reduced from 3x to 1x because the pharmacokinetics of mesotrione are similar between mice and humans and there is no indication that humans would accumulate plasma tyrosine in greater concentrations than mice. Based on these data the combined UF can be reduced from 100x to 30x for mesotrione while maintaining equivalent protection for the human health risk assessment.

2681 Human Variability in High-Throughput Risk Prioritization of Environmental Chemicals

C. L. Ring[1,2], R. W. Setzer[2], R. Pearce[2] and J. F. Wambaugh[2]. [1]*Oak Ridge Institute for Science and Education, Oak Ridge, TN and* [2]*National Center for Computational Toxicology, US EPA, Research Triangle Park, NC.*

We incorporate more-realistic human variability into an open-source high-throughput (HT) toxicokinetics (TK) modeling framework for use in a next-generation risk prioritization approach. Risk prioritization involves rapid triage of thousands of environmental chemicals, most which have little or no existing TK data. Chemicals are prioritized based on model estimates of hazard and exposure, to decide which chemicals should be first in line for further study. Hazard may be estimated with *in vitro* HT screening assays, e.g., US EPA's ToxCast program. Bioactive ToxCast concentrations can be extrapolated to doses that produce equivalent concentrations in body tissues using a reverse TK approach in which generic TK models are parameterized with 1) chemical-specific parameters derived from *in vitro* measurements and predicted from chemical structure; and 2) with physiological parameters for a virtual population. Here we draw physiological parameters from estimates of distributions of demographic and anthropometric quantities in the modern US population based on the most recent CDC NHANES data. A Monte Carlo approach, accounting for the correlation structure in physiological parameters, is used to estimate ToxCast equivalent doses for the most sensitive portion of the population. For risk prioritization, ToxCast equivalent doses are compared to estimates of exposure rates based on Bayesian inferences drawn from NHANES urinary analyte biomonitoring data. The inclusion of more-realistic human variability in the TK modeling framework allows targeted risk prioritization for demographic groups of interest. (This abstract does not necessarily represent US EPA policy.)

2682 Uncertainty Factors to Account for Interindividual Pharmacokinetic Variability in Patient Populations for Acceptable Daily Exposure and Cleaning Validation Limit Calculations

A. J. Streeter[2] and E. C. Faria[1]. [1]*Janssen Research & Development LLC, Johnson & Johnson, Raritan, NJ and* [2]*Janssen Research & Development LLC, Johnson & Johnson, Spring House, PA.*

The elderly constitute a significant, potentially sensitive, subpopulation within the general population, which must be taken into account when determining an acceptable daily exposure (ADE) for the purpose of cleaning validations for pharmaceuticals. Known differences in the pharmacokinetics of drugs between young adults and the elderly are potential contributors affecting the interindividual uncertainty factor (UFH) component of the ADE calculation. The UFH values were calculated for 206 drugs for young and elderly groups separately and combined (with the elderly assumed to be a sensitive subpopulation) from published studies where the pharmacokinetics of the young and elderly groups were directly compared. For the young adult data, which could be considered to be equivalent to a healthy worker population, 100% of the drugs demonstrated UFH values <10, while for the combined data, 95% of the drugs demonstrated UFH values of <15. Based on the analysis presented here, it is recommended to use a default UFH value of 10 for worker populations where no data exist, while it is recommended to use a default UFH value of 15 for the general population. The underlying reasons for the large differences between the exposures in the young and elderly subjects for the 10 compounds which show the greatest separation are different in almost every case, involving the OCT2 transporter, glucuronidation, hydrolysis, CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP3A4 or CYP3A5. Therefore, there is no consistent underlying mechanism which is responsible for the largest differences in pharmacokinetic parameters between young and elderly subjects.

2683 Relationship Between LD50s and LOAELs for Acute, Intermediate, and Chronic Oral Exposures

R. C. Siwakoti[1,2], E. Demchuk[2], A. J. Prussia[2] and C. J. Welsh[2]. [1]*Georgia Institute of Technology, Atlanta, GA and* [2]*Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, Atlanta, GA.* Sponsor: P. Ruiz.

Median lethal dosage (LD50) is the most abundant endpoint in mammalian toxicology. The ability to extrapolate from LD50 to a low-dose Health Guidance Value (HGV) is appealing when the HGV does not exist. In the present work, a statistical relationship between rat oral LD50 and the Lowest Observed Adverse Effects Level (LOAEL) was investigated. LOAELs were extracted from the ATSDR database of Minimal Risk Levels (MRLs). The database represents a unique collection of data concerning low-dose adverse health effects classified by exposure duration. Using it, LOAELs specific to acute, intermediate, and chronic exposure durations were determined. The strength of association between log-LOAELs and log-LD50s in molar units was evaluated using correlation and regression analyses. These tests showed that LD50 was the most strongly correlated to acute LOAELs, with the correlation coefficients of 0.81, 0.71, and 0.66 for acute, intermediate, and chronic LOAELs, respectively. In any studied LOAEL group, linear regression analysis did not identify a slope coefficient statistically different from unity. Consequently, it was valid to derive surrogate LOAELs from LD50s by scaling. Median LD50/LOAEL quotients suggested scaling factors of 20, 25, and 40 for derivation of acute, intermediate, and chronic surrogate LOAELs, respectively. Surrogates obtained this way do, however, carry large uncertainty. Distributions of LD50/LOAEL quotients derived in the present work empower health-assessors to select a custom reference quantile that provides a specific health-protection level required by the exposure scenario. For example, the distributions suggest that chances for an acute, intermediate, and chronic surrogate LOAEL to be health-protective are only 36%, 32%, and 18%, respectively; if an early-day US EPA scaling factor of 10 is used. The proposed approach for evidence-based HGVs complies with the policy of the National Academy of Sciences, which in its "Silver Book" promotes the "use of distributions instead of uncertainty factors" in HGV derivation.

2684 Time Extrapolation Factors for Risk Assessment: Are Group Specific Extrapolation Factors Possible?

S. E. Escher[2], M. Pastor[3], P. Carrio[3], S. Hoffmann-Doerr[1], T. Steger-Hartmann[4] and I. Mangelsdorf[2]. [1]*Henkel AG & Co, Düsseldorf, Germany;* [2]*Chemical risk assessment, Fraunhofer ITEM, Hannover, Germany;* [3]*IMIM, Universitat Pompeu Fabra, Barcelona, Spain and* [4]*Investigational Toxicology, Bayer HealthCare, Berlin, Germany.* Sponsor: C. Dasenbrock.

In regulatory risk assessment, extrapolation factors (EFs) are used for filling data gaps, thus reducing the amount of animal testing. Extrapolation is common praxis to consider i.e. time-, interspecies-, intraspecies extrapolation. Time extrapolation is performed in cases where a short time study is available, but safety assessment for chronic exposure conditions is required. The NO(A)EL (no observed (adverse) effect level) of the long term study is then estimated by applying the corresponding extrapolation factor. In the present project, time extrapolation factors for oral administration routes are determined by a probabilistic approach. Paired studies with oral exposure for the same chemical, testing the same species but with different study durations were extracted from literature and several toxicological databases (DB), namely the ECHA CHEM, ToxRef, IMI Etox , ELINCS and Fraunhofer RepDose DB. Datasets were compiled for the extrapolations of subacute to subchronic and of subchronic to chronic. Probabilistic analyses were carried out with the resulting two EF-datasets. In the largest dataset subchronic to chronic, which comprises more then 1000 individual EFs, the possibility to derive EFs for classes of compounds using statistical methods (like ANOVA or regression analysis) has been evaluated. Classes were built according to potency, physico-chemical as well as structural properties. The analysis of subgroups with different toxicological potency revealed a consistent trend in both datasets. Compounds with relatively low NOELs in the short term study showed on average significantly lower time EFs compared to those being less toxic. For subchronic to chronic an overall time EF of 1.8, for subacute to subchronic of 1.6 is derived. Both EFs are lower as compared to the current EF of 2 and 3 recommended by ECHA.

2685 Target Organ Profiles in Toxicity Studies Supporting Human Dosing: Does Severity Progress with Longer Duration of Exposure?

R. Roberts[1], R. Callander[2], P. Duffy[1], R. Knight[1], M. Jacobsen[2] and A. Boobis[3]. [1]*ApconiX, Alderley Edge, United Kingdom;* [2]*AstraZeneca, Alderley Edge, United Kingdom and* [3]*Imperial College, London, United Kingdom.*

We have previously reported the profile of target organs (defined as organs showing histopathological changes) in rodent and non-rodent toxicity studies conducted prior to first-time-in-man (FTiM) for 77 AstraZeneca candidate drugs (CDs). Here, we test the assumption that toxicity is exacerbated by dosing duration by comparing the incidence and severity of target organ toxicities in these ≤6 week FTiM studies with those observed in subsequent subchronic/chronic (≥3 month) studies. Looking at the effect of dosing duration on severity (pathological score) and incidence (percentage of animals within the group) for the 39 CDs that met the criteria for inclusion (comparable doses between FTiM and subchronic/chronic studies), new toxicities appeared for 31 target organs but existing ones resolved for 29 target organs. Increased severity was more frequent for rodent (16 target organs) than for non-rodent (4 target organs. Most notable changes were a large increase in severity/incidence in liver and in non-rodent lung in contrast to a large decrease in severity and incidence for kidneys/ureter and for the non-rodent thymus. Overall this analysis shows that, even with continued exposure, target organ toxicities of CDs are as likely to show partial or complete recovery as they are to progress in severity. These findings are surprising and suggest a much more complex picture than previously assumed. For the key target organs studied here, there appeared to be a high level of adaptation to prolonged exposure for some CDs and in some tissues, but without a clear pattern that could form the basis of a predictive model at the present time. This has great significance for human risk assessment with differing potential impact depending on the sphere of toxicology.

2686 Use of the Collaborative Cross Mouse Panel to Assess the Effect of Genetic Variability in the Low-Dose Region of a TCDD-Induced Dose-Response Curve

P. Dornbos[3], M. Warren[4], R. Crawford[3], N. Kaminski[3], D. Threadgill[4] and J. J. LaPres[3]. [1]*Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI;* [2]*Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI;* [3]*Environmental and Integrative Toxicological Sciences, Michigan State University, East Lansing, MI and* [4]*Interdisciplinary Program in Toxicology, Texas A&M University, College Station, TX.*

Traditional lab-based toxicologicy studies do not typically account for inter-individual variability in the human population and, thus, lack the means to accurately assess the potential risk of chemical exposures. Notably, as chemical exposures occur in heterogeneous populations, lab-based toxicology is somewhat disconnected from risk management decision-making. A recent report from the National Research Council (NRC) hypothesized that the shape of the low-dose region of a non-cancer causing dose-response relationship (DRR) would effectively linearize when accounting for the inter-individual variability in the human population. Given the direct implications of the NRC's hypothesis in risk-management, we propose to assess the knowledge gap with an *in vitro* screen assessing a 2,3,7,8-tetracholorodibenzo-p-dioxin (TCDD)-induced dose-response of CD40 ligand activated primary B cells isolated from 36 strains of the collaborative cross (CC) mouse panel. The CC consists of a large number of inbred mouse strains that encompass the genetic variability of the human population. Utilizing several of the parental strains of the mouse panel, preliminary results suggest that TCDD induces a suppressive effect in two distinct endpoints: 1) the number of cells secreting IgM and 2) the concentration of IgM secreted. More importantly, these strains displayed differing sensitivity to TCDD, providing proof of concept that our model encompasses inter-individual variability between strains. Ultimately, the DRRs from the mouse panel will be used to statistically model the low-dose region; such results have potential to address the effect of population-level genetic variability in the low-dose region of a receptor-mediated DRR.

2687 Human Inter-Individual Variability Does Not Linearize the Low-Dose Region of a TCDD-Induced Dose-Response Curve in Primary B Cells

P. Dornbos[3], R. Crawford[3], N. Kaminski[3] and J. J. LaPres[3]. [1]*Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI;* [2]*Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI and* [3]*Environmental and Integrative Toxicological Sciences, Michigan State University, East Lansing, MI.*

The effect of inter-individual variability is not typically assessed in traditional toxicological studies. Given that chemical exposures occur in heterogeneous populations and inter-variability will impact accurate risk assessment, this knowledge gap has the potential to negatively impact public health and industrial and municipal finances. A recent report from the National Research Council (NRC) suggests that, when accounting for inter-individual variation in responses, traditionally assumed nonlinear dose-response relationships (DRRs) would better be explained with a linear model within the low-dose region for non-cancer causing endpoints. Notably, the NRC's hypothesis is not supported by adequate data. To directly test the NRC's hypothesis, this project focused on assessing the DRR between 2,3,7,8-tetracholorodibenzo-p-dioxin (TCDD) exposure and immune suppression in a cohort of human donors. Human B cells were isolated from 50 unique donors, activated with CD40 ligand, and exposed to increasing levels of TCDD on a logarithmic scale (0 through 30 nM TCDD). Two endpoints sensitive to TCDD were assessed: 1) number of IgM secreting B cells; and 2) amount of IgM secreted. The results indicate that increasing doses of TCDD have a significant suppressive effect on both endpoints (p value < 0.05). More importantly, the low-dose regions of the DRRs were statistically modeled and comparison of model results suggest that the low-dose region of the DRRs from both endpoints fit better to a nonlinear statistical model as opposed to a linear model. Such results have potential to better inform risk-management decision-making for not only TCDD, but for other receptor-mediated non-cancer causing endpoints as well.

2688 Chronic Exposure Induces Adverse Effects or Adaptive Responses? Lessons Learned from Radiotoxicology Studies to Low Doses

Y. Gueguen, K. Tack and M. Benderitter. *PRP-HOM/SRBE, IRSN, Fontenay-aux-Roses, France.* Sponsor: M. Pallardy.

Knowledge of health risk for chronic exposure to low doses of stressors is of essential societal issue for occupational workers or general populations at risk of exposure due to anthropogenic activities or natural exposure. Research conducted in the field of radiotoxicity or radiobiology to low doses must contribute to the radiation protection of workers and populations to limit the risk of exposed populations. Despite the existence of this system, many uncertainties still exist today regarding the quantification of health effects of exposure to ionizing radiation, especially chronic exposure to low doses. The Linear No Threshold (LNT) model relates dose to effect in a linear fashion that has been extrapolates from high doses effects. However, numerous studies these last 25 years, among different organisms from bacteria to human beings, have shown that non-monotonic dose-response curve (NMDR) occurs for dose-response exposure: (1) U-shaped (or adaptive response) which admits the ability of an organism or a living cell to "adapt" to low intensity of stress and (2) inverted U-shaped (or supra-linear response) admits an "additive" effect to low doses based on the existence of "by-stander" effect. Several reports were published on the effects of low doses of ionizing radiation (BEIR VII, UNSCEAR, ICRP, and French Academy of Sciences) that led to paradigm shifts in radiobiology. Although most mechanistic studies were designed to study cancer effects, the non-cancerous effects of low doses have been more recently documented. In this context, IRSN through its ENVIRHOM research program obtained experimental results allowing the identification of new tissue targets and non-cancerous effects based on the radionuclide type and exposure. It is important to note, that at low doses, they induce no deleterious effect which suggest that adaptive response could occurs. Several hypotheses may explain this phenomenon, including notably one based on the activation of normally silent genes for the expression of DNA repair enzyme and a second based on the induction of enzymes capable of removing toxic agents generated by the cell when it receives a certain dose of radiation.

2689 Development of a Screening Framework for Pesticide Cumulative Risk Assessment

M. Perron, A. Lowit, C. Smith and D. Wilbur. *US EPA, Washington, DC.*

As part of the Federal Food, Drug, and Cosmetic Act (FFDCA), the US Environmental Protection Agency (EPA) is required to take into account available evidence concerning the cumulative effects of pesticides and other substances that have a common mechanism of toxicity. Currently, the EPA's Office of Pesticide Programs (OPP) has guidance documents (www.epa.gov/oppsrrd1/cumulative) that describe the process for establishing groups of chemicals with a common mechanism of toxicity (i.e., common mechanism groups (CMGs)) and the steps used in conducting a cumulative risk assessment (CRA); however, these documents do not provide guidance on how to screen and/or prioritize the large number of registered pesticides that need to be evaluated. Recently, a screening framework for CRAs has been developed by EPA-OPP aiming to assist scientists and decision-makers in screening pesticides for potential CMGs and conducting screening-level CRAs. The screening analysis described in this framework begins with a weight-of-evidence analysis of the toxicological knowledgebase to determine if the available evidence for a particular group of pesticides does or does not support common toxicological profiles. If a candidate CMG can be formed, then the screening process continues by using tiered approaches to evaluate dietary, residential, and aggregate exposure with each tier providing a higher level of refinement. Application of the screening framework and analyses conducted thus far for two case studies (sulfonylurea herbicides and mectin insecticides) will be presented. This screening-level approach will allow the EPA to efficiently use resources to address the FFDCA requirements concerning cumulative effects of pesticides with a common mechanism of toxicity.

2690 Implementation of the Screening Framework for the Cumulative Risk Assessment of Pesticides

E. Craig, M. Perron, H. Pope-Varsalona and A. Lowit. *US EPA, Washington, DC.*

The EPA's Office of Pesticides Program (OPP) recently published a guidance framework for establishing candidate common mechanism groups (CMGs) and conducting screening cumulative risk assessments (CRA). The screening analysis for CRA described in this guidance consists of a two-tier approach that begins with an evaluation of the toxicological knowledgebase available on a particular group of pesticides followed by risk-based screening. In order to implement this framework, potential groups of pesticides that can be subjected to screening must first be identified. However, no single repository of pesticides currently exists that groups these chemicals based on their mode of action (MOA)/adverse outcome pathways (AOPs) and/or structural similarity. Here, an approach is described that identifies potential pesticide groups utilizing a general list of pesticides based on pesticidal activity (www.alanwood.net/pesticides/class_pesticides.html) and curating this list based on pesticide registration in the United States. The initially identified pesticide groups are prioritized taking into account the US pesticide registration and re-registration schedule. Next, prioritized pesticide groups are subjected to screening analysis using structural and toxicological information from publically available sources (i.e. DSSTox) as well as data provided for pesticide registration. As a starting point, eleven potential groups have been identified, some of which have specific target organs and potential common mechanisms of toxicity. In the case of groups with general liver toxicity (i.e. changes in liver weight but no other effects), tools such as ToxCAST will be applied to support the screening process. Overall, this prioritization effort will allow EPA to address the need to conduct CRAs while efficiently employing currently available platforms such as DSSTox and ToxCAST.

2691 Web-Based Calculator for Vapor Intrusion Screening Levels (VISL)

L. Galloway[3], F. Dolislager[3], S. Smith[3], A. M. Frame[2] and L. G. Gaines[2]. [1]*The University of Tennessee, Knoxville, Knoxville, TN;* [2]*Office of Superfund Remediation & Technology Innovation Science Policy Branch, US EPA, Washington, DC and* [3]*The Institute for Environmental Modeling, The University of Tennessee, Knoxville, Knoxville, TN.* Sponsor: C. Bast.

The U.S. Environmental Protection Agency Office of Remediation and Technology Innovation (OSRTI), through an interagency agreement with Oak Ridge National Laboratory (ORNL), has developed an online calculator for vapor intrusion screening levels (VISL). This tool provides screening level concentrations for groundwater, soil gas (sub-slab and exterior), and indoor air for certain qualifying chemicals to assist risk assessors in determining risks due to vapor. The screening levels for groundwater and soil gas (either sub-slab gas or soil gas collected exterior to buildings) are calculated from the target indoor air concentrations using empirically-based conservative "generic" attenuation factors that reflect generally reasonable worst-case conditions as described in the EPA's draft vapor intrusion guidance (EPA 2002). The default, generic VISLs are based on default exposure parameters and factors that represent Reasonable Maximum Exposure (RME) conditions for long-term/chronic exposures. In addition to calculating screening levels, this tool can calculate indoor air concentrations from soil gas and groundwater concentrations entered by the user. The risks from calculated indoor air concentrations and user-provided indoor air concentrations can also be calculated. This new tool will be useful for concerned citizens, risk assessors and risk managers.

2692 Chemical Alternative Assessment: Where Do I Start?

E. Freeman[1] and J. Nusz[2]. [1]*Center for Chemical Regulation and Food Safety, Exponent, Pittsburgh, PA and* [2]*EcoSciences, Exponent, Boulder, CO.*

A chemical alternatives assessment is designed to find "safer" alternatives to a "chemical of concern". The most important part of the alternatives assessment is the scoping and problem formulation phase; all aspects to be considered in the alternatives assessment are identified and defined at this phase. The key stages of the alternatives assessment are the evaluation of the physical and chemical properties, relative exposure, human health hazards and ecological hazards of the alternatives. The selection of the endpoints within those parameters, the ranking system used to assess those data , and the process for integrating potential exposure and hazard to predict the risk of the alternatives must also be defined in the scope and problem formation phase of the assessment. Other parameters, such as lifecycle assessment and cost and feasibility assessments, should also be considered and can be used to further guide the assessment in order to ensure selection of the best possible alternative to the chemical of concern. The presentation is designed to provide an understanding of the importance of the scope and problem formulation stage of an alternative assessment, what decisions and information gathering are necessary prior to beginning the alternative assessment, where data can be obtained for an alternatives assessment, and what options are available for ranking and integrating those data.

2693 Green Jobs: Definition and Method of Appreciation of Chemical and Biological Risks

J. Zayed[1,2], E. Cheneval[2], M.-A. Busque[1], C. Ostiguy[1,2], J. Lavoie[1,2], R. Bourbonnais[2], B. Bakhiyi[2] and F. Labrèche[1,2]. *[1]Robert-Sauvé Research Institute of Occupational Health and Safety (IRSST), Montreal, QC, Canada and [2]Department of Environmental and Occupational Health, University of Montreal, Montreal, QC, Canada.*

Within a sustainable development approach, the "green economy" emerges as an enlightened vision for reconciling the environment and the economy. It gives rise to the concept of green jobs that have grown fast and changed the work environment. However noble and ambitious they are, green jobs are not automatically safe. Besides providing a clear definition of green jobs, the aim of the study was to conduct a preliminary risk assessment of chemical and biological agents for workers in Quebec. Thus was set an operational definition, encompassing criteria and sustainable development principles in order to establish a list of green job titles. Chemical and biological risks associated with those selected green jobs were assessed, using an innovative and adapted assessment method based on a control banding approach. A double entry model, treating chemical contaminants and biological agents separately, allowed us to prioritize green job titles according to the level of chemical and biological risks. Twenty one high-risk jobs were identified. They are related to three sectors: waste management, energy and green products and substitute materials. The developed method is flexible and could be an applicable tool to better apprehend the risks for workers and to put forward faster and more suitable control measures.

2694 Characterization of Chemical-Induced Asthma Risk: Hazard and Exposure Assessment Approaches

M. Vincent, A. Maier, A. Parker and B. Gadagbui. *Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH.*

Asthma is a complex syndrome with multiple etiologies, which complicates our understanding of the exposure-disease pathway. Concerns have been raised regarding the potential for consumer products to cause or exacerbate asthma or asthma-like responses. However, risk assessments are hampered by limitations in both epidemiology and toxicology methods. To address this challenge, a decision system was developed that includes sequential data analysis techniques. First, a hazard characterization process employing a weight of evidence basis for assessing the current human effects and animal toxicology literature was used to identify current gaps in our understanding of asthma and relationships with cleaning product use by applying the principle of Systematic Review. The results of this characterization process served as a key input for a hazard-based prioritization approach that stratifies chemicals by the likelihood of concern for asthma responses based on effect ratings for two toxicological endpoints: irritation and sensitization. High priority compounds are then flagged for a more robust tiered safety assessment. A parallel structure is employed to include dose-response methods for asthma endpoints and methods for scenario specific exposure estimation; these tracks are integrated in a risk characterization step. A tiered assessment structure is used to accommodate different amounts of data for both the dose-response assessment (i.e. use of existing benchmarks, hazard banding, or the threshold of toxicological concern) and exposure estimation (i.e., use of empirical data, model estimates, or exposure categories). A case study for acetic acid was developed to test the safety assessment methodology. Key gaps and testing needs that we further explored include: methods for modeling exposure to aerosols, tools for using mode of action data to inform relative importance of irritant and sensitization data for asthma, and the absence of existing inhalation-based exposure guidelines pertinent to asthma.

2695 Analysis of the Indirect Genotoxic Effects of Asbestos: Implications for Dose Response Models

A. K. Madl, A. Bernal, D. M. Cowan, M. Liong, N. Boulos, C. Poteete and M. Kovochich. *Cardno ChemRisk, Aliso Viejo, CA.*

Asbestos fiber toxicology and the risk of malignant disease is dependent on fiber dose, type, and dimension. Although the precise mechanistic pathways by which asbestos fibers cause a tumorigenic response have not been elucidated, one of several proposed mechanisms is through asbestos-induced genotoxic and mutagenic effects. While the historical regulatory approach to dose-response modeling of DNA-reactive compounds has been the linear no-threshold cancer model, alternative threshold models have gained some acceptance when the appropriate evidence for a threshold mode-of-action (MOA) exists. To evaluate the available evidence for an indirect vs. direct MOA for asbestos, this analysis critically assessed over 130 genotoxicity *in vivo* and *in vitro* studies of asbestos fibers with respect to: 1) assays utilized to address an indirect vs. direct MOA, and 2) assay dose and fiber type used in relation to relevant human equivalent doses in occupationally-exposed individuals. Most *in vivo* studies were not informative to address the question of an indirect vs. direct MOA. Of the 46 *in vitro* studies that evaluated indirect vs. direct effects, the overwhelming evidence suggests that the genotoxic action of asbestos fibers occurs through indirect mechanisms, such as ROS generation. Additionally, the human equivalent doses utilized in the *in vitro* and *in vivo* genotoxicity assays were several orders of magnitude greater than the median and upper bound fiber lung burdens observed in heavily exposed workers (253,000 and 8,540,000 f/g, respectively), as well as over 11,000-fold greater than lung fiber burdens found in individuals with pleural plaques. This analysis provides a current weight-of-evidence for the genotoxic MOA of asbestos fibers and supports the role of cellular thresholds for indirect asbestos-induced genotoxic effects. This study provides critical information for risk assessors to form a basis for selection of dose-response models that attempt to understand carcinogenic risks to asbestos at low levels of exposure.

2696 Smoker's Risk of Lung Cancer from Asbestos Exposure

N. Moore, B. Hardin, C. Robbins and B. Kelman. *Veritox, Inc., Redmond, WA.*

Cigarette smoke and asbestos have been established as causes of lung cancer and synergistic interactions between cigarette smoke and asbestos were characterized in the mid-twentieth century. Since then, cigarette smoke has emerged as the primary cause of lung cancer; smoking exposure parameters for risk assessments have evolved; cigarette smoke has been identified as a more potent lung carcinogen than asbestos; and exposures to asbestos and cigarette smoke have declined dramatically. Original cohorts used to evaluate the asbestos-smoking interaction originated prior to 1922 through approximately 1965, represent exposures of 150-600 f-yr, and smoking histories in excess of 40 pack-yr. A greater-than-additive rate of lung cancer is evident in many of these studies although the exact magnitude of the synergy varies by study, likely due to a lack of quantitative measures of both asbestos and smoking. Today, cohorts are no longer comparable to those previously studied because current occupational lifetimes occurred during periods of declining asbestos use, increasing asbestos exposure regulations, and declining smoking prevalence. For example, construction workers with a 15-year history of working with asbestos-containing materials (1986–2001) have exposures corresponding to approximately 1.4 f-yr. We conducted an analysis of studies containing cohorts with quantitative or semi-quantitative measures of asbestos and smoking exposures after 1972. An exhaustive literature search identified a single peer-reviewed publication with semi-quantitative exposure information for both smoking and asbestos. A quantitative assessment was conducted by combining job classifications from the identified study with exposure measurements from additional publications, which introduced considerable uncertainty. Nevertheless, results indicate lung cancer risk attributable to asbestos in smokers is insubstantial until both asbestos and cigarette smoke cumulative exposures are high. The implication of these data is that lesser asbestos exposures are not associated with increased risk in lung cancer among smokers.

2697 Assessment of the *In Vivo* Genotoxicity of CrVI in Target Organs Identified in a Two-year Cancer Bioassay

C. M. Thompson[2], M. Suh[3], D. M. Proctor[3], J. E. Rager[1], L. C. Haws[1] and M. A. Harris[2]. *[1]ToxStrategies, Austin, TX; [2]ToxStrategies, Katy, TX and [3]ToxStrategies, Mission Viejo, CA.*

Studies indicate there is strong correlation between *in vivo* genotoxic potency and *in vivo* carcinogenic potency. This correlation exists despite the fact that genotoxicity is often measured in non-target tissue (i.e. those that do not develop tumorigenic responses). Here, we review the *in vivo* genotoxicity of hexavalent chromium (CrVI) in drinking water in target and non-target tissues. In a 2-yr study, CrVI in drinking water increased duodenal adenomas and carcinomas in B6C3F1 mice at ≥20 ppm, and oral cavity squamous cell carcinomas in F344 rats at ~180 ppm. Results for peripheral blood micronucleus (MN) assays in the literature were generally negative. In the gastrointestinal tract, we measured mutant frequency (MF) in the oral mucosa of Big Blue® TgF344 rats. Relative to controls, a statistically significant (20- to 40-fold) increase in cII MF was observed in rats treated with 10 ppm 4NQO, but no change

occurred in rats treated with 180 ppm CrVI. In the duodenum of B6C3F1 mice, we measured a high background MF of k-ras codon 12 GAT mutations in control mice (~1E-3); however, no differences in MF were observed in mice treated with 180 ppm CrVI. Several analyses of duodenal crypts failed to indicate an increase in crypt MN in B6C3F1 mice exposed to 180 ppm CrVI. Phosphorylated H2AX immunostaining corresponded to aberrant crypt enterocytes in cyclophosphamide-treated mice, but not CrVI-treated mice. Consistent with these negative findings, X-ray fluorescence microscopy demonstrated Cr fluorescence in duodenal villi but little or no Cr fluorescence in duodenal crypts. Transcriptomic analyses did not indicate activation of signaling commonly involved in intestinal carcinogenesis (e.g. Wnt/β-catenin signaling). In summary, the low systemic genotoxic potency of CrVI was consistent with the lack of systemic carcinogenic potency; and genotoxic responses were negative in site of contact tumor tissues. The absence of genotoxic responses in target tissues suggest that non-genotoxic mechanisms are likely involved in the tumorigenic responses.

PS 2698 Re-Evaluation and Classification of Duodenal Lesions in B6C3F1 Mice and F344 Rats from Four Studies of Hexavalent Chromium in Drinking Water

J. M. Cullen[3], J. M. Ward[1] and C. M. Thompson[2]. [1]*Global VetPathology, Montgomery Village, MD;* [2]*ToxStrategies, Katy, TX and* [3]*College of Veterinary Medicine, North Carolina State University, Raleigh, NC.*

Thirteen-week and 2-year drinking water studies conducted by the National Toxicology Program (NTP) reported that hexavalent chromium [Cr(VI)] induced diffuse epithelial hyperplasia in the duodenum of B6C3F1 mice but not F344 rats. In the 2-year study, Cr(VI) exposure was additionally associated with duodenal adenomas and carcinomas in mice only. Subsequent 13-week Cr(VI) studies conducted by another group demonstrated non-neoplastic duodenal lesions in B6C3F1 mice similar to those of the NTP study, as well as mild duodenal hyperplasia in F344 rats. Because intestinal lesions in mice are the basis for proposed safety standards for Cr(VI), and the histopathology data are relevant to the mode of action, consistency (an important Hill criterion for causality) was assessed across the aforementioned studies. Two veterinary pathologists applied uniform diagnostic criteria to the duodenal lesions in rats and mice from the four repeated-dose studies. Comparable non-neoplastic intestinal lesions were evident in mice and rats from all four studies; however, the incidence and severity of intestinal lesions were greater in mice than rats. These findings demonstrate consistency across studies and species, and highlight the importance of standardized nomenclature for intestinal pathology. The differences in the severity of non-neoplastic lesions also likely contribute to the differential tumor response.

PS 2699 Evaluation of the Human Relevance of Bladder Lesions Induced by Dietary 2-phenylphenol in Male Rats

P. R. McClure[1], K. Zaccaria[1], M. A. Odin[1], L. E. Lizarraga[2] and Q. J. Zhao[2]. [1]*SRC, Inc., North Syracuse, NY and* [2]*NCEA, US EPA, Cincinnati, OH.*

Chemical human health hazard identification often relies on animal toxicity data. Establishing the relevance of health effects observed in experimental animals to humans is important for risk assessment. In the present analysis, mechanistic evidence was evaluated to determine the human relevance of potential modes of action (MOA) for urinary bladder tumors following exposure to 2-phenylphenol (2PP). Subchronic or chronic exposure to ≥1% dietary concentrations of 2PP or its sodium salt (Na2PP) produced epithelial hyperplasia and tumors in the urinary bladder of male rats and, less frequently, female rats. In contrast, urinary bladder lesions were not found in mice, hamsters or guinea pigs similarly exposed. Data show that cytotoxicity, epithelial regeneration, micronuclei, and DNA strand breaks in the bladder were only observed at dietary concentrations that were also associated with appearance of lesions. Based on weight of evidence, two key events are proposed in MOA hypotheses for 2PP-induced bladder lesions in the male rat: 1) cytotoxicity and sustained regenerative cell proliferation, and 2) dose-dependent genotoxic actions of reactive metabolites of 2PP. These two key events are plausible in humans. Rats, mice, and humans have similar metabolic pathways for 2PP, and minor metabolic differences between mice and rats do not account for differences in susceptibility. Likewise, comparison of pH, osmolality, and crystal formation in urine from rats, mice, guinea pigs, and hamsters fed 2% Na2PP in the diet failed to find differences associated with susceptibility. Taking into account kinetic and dynamic factors, there is inadequate evidence to explain species differences in bladder toxicity in rodents; therefore, it is reasonable to conclude that 2PP-induced male rat urinary bladder lesions may be relevant to humans. The views expressed in this abstract are those of the authors and do not necessarily reflect the views and policies of the US EPA.

PS 2700 Comparison of α2u-Globulin Nephropathy Induced by ETBE and Tert-Butanol Using PBPK Modeling

K. D. Salazar[2], J. S. Lee[2], C. J. Brinkerhoff[2] and W. A. Chiu[1]. [1]*Texas A&M University, College Station, TX and* [2]*US EPA, Washington, DC.*

Renal tubule tumors that develop as the result of α2u-globulin nephropathy are specific to male rats and are not considered to be relevant for human health risk assessment. Ethyl-tertiary butyl ether (ETBE) and tert-butanol (TBA) are two gasoline oxygenates that induce similar noncancer kidney effects and share similar metabolic pathways in rats. The databases for ETBE and TBA regarding α2u-globulin nephropathy both contain evidence of accumulating α2u-globulin in the hyaline droplets but differ with respect to subsequent steps in histopathological sequence such that only TBA induced renal tubule hyperplasia, granular casts, and renal tumors. The similar metabolism of these two chemicals provides an opportunity to examine the consistency of α2u-globulin nephropathy endpoints on an internal dose basis across two chemical databases. We used physiologically based pharmacokinetic (PBPK) modelling to determine if the α2u-globulin-associated endpoints are consistent across ETBE and TBA studies when using TBA blood concentration as the dose metric. The induction of hyaline droplets, α2u-globulin, granular casts, tubular hyperplasia, and renal tumors were not consistent across chemical and route of exposure when compared on an internal dose basis. These findings suggest that other experimental variables besides internal TBA concentrations may play a role in α2u-globulin-mediated nephropathy and renal tumors. This work demonstrates the utility of applying PBPK modeling to understand the mode(s) of action for these two chemicals. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.

PS 2701 Application of the Adverse Outcome Pathway (AOP) Approach to Inform Mode of Action (MOA): A Case Study with Inorganic Arsenic

H. Clewell[3], J. Yager[1], T. Greene[2] and R. Gentry[2]. [1]*Environ Ramboll, Emmeryville, CA;* [2]*Environ Ramboll, Monroe, LA and* [3]*Environ Ramboll, Raleigh, NC.*

Adverse outcome pathways (AOPs) are intended to reflect chemical-independent descriptions of the series of key events linking a particular molecular initiating event (MIE) with a specified adverse outcome (AO). Modes of action (MOAs), on the other hand, are chemical-specific and include explicit consideration of metabolism and dose-response as they relate to production of a particular adverse outcome. In this case study, we describe the process of deriving MOAs for the cancer and noncancer effects reported following exposure to inorganic arsenic based on a number of AOPs related to disruption of cellular signaling by chemicals that bind to protein thiols. The proposed MOAs for inorganic arsenic superimpose highly-specific direct interactions with critical proteins containing vicinal dithiols, overlaid on a background of oxidative stress that induces an array of responses common to many other types of cellular stressors. The most challenging aspect of developing MOAs from AOPs is the incorporation of dose-response. In the case of inorganic arsenic, the cellular responses to low concentrations appear to be essentially adaptive, characterized by disruption of inflammatory/survival signaling coupled with an apparent over-response to oxidative stress. At higher concentrations, induction of genes associated with proliferation reflects a cellular response to toxicity, while increased expression of cell cycle checkpoint control genes suggests cellular recognition of unrepaired DNA damage. At even higher concentrations, the apoptotic response dominates, reflecting the efficacy of inorganic arsenic as an anti-neoplastic agent. The observed human effects of inorganic arsenic, both cancer and noncancer, can to a large extent be explained by these dose-dependent MOAs. The proposed MOA further allows for an integrated approach to conducting a risk assessment for inorganic arsenic. (Funded by EPRI)

2702 Strength of Human Evidence for Bile Duct Cancer in Japanese Printers Exposed to 1,2-Dichloropropane

M. A. Odin[1], K. J. Zaccaria[1], P. R. McClure[1] and Q. J. Zhao[2]. [1]*SRC, Inc., North Syracuse, NY and* [2]*US EPA, Cincinnati, OH.*

A previous US EPA cancer hazard evaluation assigned 1,2-dichloropropane (1,2-DCP) to Group B2 (Likely to be carcinogenic in humans) based on sufficient evidence in animals and no human evidence. The animal evidence included equivocal evidence of mammary gland tumors in female rats and sufficient evidence of liver tumors in male and female mice in 2-year oral bioassays and sufficient evidence of nasal tumors in male and female rats, Harderian gland tumors in male mice, and lung tumors in female mice in 2-year inhalation bioassays. Recently published cancer cluster and case-series reports indicate that occupational exposure to 1,2-DCP in the Japanese printing industry may be associated with the development of cholangiocarcinoma, a rare form of bile duct cancer. The strongest association was found in a print shop in which 17/111 printers exposed to 1,2-DCP and/or dichloromethane developed cholangiocarcinomas. The rarity of this cancer in humans merits further research attention, but several limitations restrict the strength of the human evidence: (1) the evidence is from a small number of reports in a few print shops; (2) affected workers were often exposed to several other solvents demonstrated to be carcinogenic in laboratory animals; (3) exposure assessments were not available in all studies; (4) statistical adjustments for confounding variables were not conducted; 5) human evidence for dose-response and temporal relationships are lacking; and (6) mechanistic studies to investigate the biological plausibility of the link between 1,2-DCP and cholangiosarcomas or other cancer types are lacking. The views expressed in this abstract are those of the authors and do not necessarily reflect the views and policies of the US EPA.

2703 Decamethylcyclopentasiloxane (D5) Risk Assessment (RA): A Quantitative Weight of Evidence Approach (QWoE) for Liver Effects

J. Bridges[2] and P. A. Jean[1]. [1]*Dow Corning Corporation, Auburn, MI and* [2]*University of Surrey, Guildford, United Kingdom.*

Risk assessments (RA) for regulatory purposes are commonly based on: Selection of the most conservative study to identify the lead effect. Use of expert judgement to choose the 'best' study(s). What is needed are harmonised RA guidelines, for using all the relevant data, which are objective, transparent and facilitate consistency. This requires: Unambiguous definition of the hypothesis/ problem. Predetermination of the criteria to evaluate quality (Q) and strength of evidence (SoE). Assessment of Q and SoE both for all studies of potential relevance. A Quantitative Weight of Evidence methodology (QWoE) is advocated as the way forward, in particular for RA's of stressors where there is a rich data base and/or the issue is controversial. A good example is D5 for which a 2015 EU SCCS Opinion on its safety in cosmetics relies on one rat chronic oral study to identify liver effects as critical for RA purposes. This conclusion raises issues as to: whether the route of exposure, the dose regimen used and the endpoints identified are suitable to determine if the observed liver effects are both adverse and valid for human RA. A QWoE methodology has been developed to address this, which draws on all the relevant papers and reports. Important Q parameters (13) have been identified, each of which is scored from 0–4. The average Q score for each study is calculated. The following SoE parameters, each scored from 1–4 were chosen: Dose comparison with worst case human exposure, dose response relationship, effects adverse not adaptive, reversibility, magnitude of change(s) relative to controls, compatibility between endpoint findings and likely modes of action. As some studies determine multiple endpoints the following weighting of SoE was used: pathology findings> gross findings, such as liver wt> functional changes eg bile flow> biochemical changes (unless related to a known adverse mode of action). Plotting the Q vs the SoE scores allows a visualisation of the extent of support that liver changes provide a suitable basis for the RA. The QWoE findings do not support the route of exposure and the liver effects as relevant for assessing the risk to consumers from D5 in cosmetics. Sponsored by Silicones Environmental, Health and Safety Center (SEHSC).

2704 Mode of Action and Human Relevance Evaluation of Dibutyl Phthalate (DBP)-Induced Male Reproductive System Toxicity

X. Arzuaga[2], T. Walker[2], G. Cooper[2] and A. Hotchkiss[1]. [1]*ORD, US EPA, Research Triangle Park, NC and* [2]*ORD, US EPA, Washington, DC.*

In vivo studies using various rat strains have shown that dibutyl phthalate (DBP), a phthalate ester used as a plasticizer, alters normal development of the male reproductive system by disrupting androgen synthesis during the masculinization programming window. However, studies using human xenograft models (fetal testicular tissue implanted in rodent hosts) report that DBP exposure does not affect androgen synthesis in developing male human fetal tissue. These findings question the relevance of the rat model for assessment of potential DBP-induced male reproductive effects in humans. In the current work, a mode of action (MOA) framework was used to evaluate the available evidence from *in vivo*, *in vitro*, and xenograft studies on DBP-induced toxicity to the male reproductive system. The proposed framework relies on established biochemical, molecular, cellular, and endocrine mechanisms which control gestational and post-natal development of the male reproductive system. Experimental studies on DBP-induced male reproductive effects were evaluated for biological plausibility as well as consistency across different species and life stages. Three conclusions were identified: 1) rats appear to be more responsive than other rodent species to DBP-induced anti-androgenic effects during gestation, 2) androgen-independent adverse responses to fetal DBP exposure are conserved among different mammalian experimental models (including rats) and human xenografts, and 3) anti-androgenic effects induced by DBP are conserved in different rodent species and non-human primates when exposure occurs during post-natal life stages. This analysis also identified data gaps, such as additional information on the specific molecular targets for DBP which activate cellular responses and lead to adverse male reproductive outcomes. Considering the similarities in male reproductive effects caused by various phthalates, the resulting MOA analysis for DBP may be useful in the evaluation of toxicological and mechanistic data on other phthalates. The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

2705 Evaluation of Butyl Benzyl Phthalate (BBP)-Induced Male Reproductive Effects; Application of a Mode of Action (MOA) Framework Developed for Dibutyl Phthalate (DBP)

X. Arzuaga[2], T. D. Walker[2], G. Cooper[2] and A. Hotchkiss[1]. [1]*US EPA, Research Triangle Park, NC and* [2]*US EPA, Washington, DC.*

Experimental studies using rats have shown that gestational exposure to BBP, a phthalate ester used as a plasticizer for various applications, disrupts testicular androgen synthesis. However, xenograft studies (fetal testicular tissue implanted in rodent hosts) using DBP suggest that the human fetal testis is resistant to phthalate-induced disruption in androgen synthesis. A mode of action (MOA) framework was used to evaluate the available experimental evidence on DBP-induced male reproductive effects. Application of this framework revealed that rats are more sensitive to phthalate-induced anti-androgenic effects during gestation, but that androgen-independent effects are conserved across species. Furthermore, androgen-dependent and -independent responses are common to different animal species when exposure occurs during early post-natal life stages. In the current work, the MOA analysis developed for DBP was used to evaluate the available experimental studies on BBP-induced effects in the male reproductive system. The evidence from studies using various rat strains suggests that BBP exposure induced both androgen-dependent and androgen-independent responses in developing rat embryos, and androgen-dependent responses when exposure occurred during post-natal life stages. Application of this MOA framework also highlighted various data gaps associated with BBP action, including incomplete evidence for androgen-independent responses after post-natal exposure, and the identification of molecular targets that are involved in activating cellular responses leading to adverse male reproductive effects. The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

2706 Human Relevance of Constitutive Androstane Receptor (CAR)-Mediated Rat Hepatocellular Tumors by Fluxapyroxad

C. Werner[3], M. Goettel[3], N. Honarvar[3], A. Vardy[2], B. Elcombe[2], C. Elcombe[2], A. M. Doi[1] and I. Fegert[3]. *[1]BASF Corporation, Research Triangle Park, NC; [2]CXR Biosciences Ltd, Dundee, United Kingdom and [3]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany; [4]Regulatory Toxicology Crop Protection, BASF SE, Ludwigshafen, Germany.*

The fungicide Fluxapyroxad (Xemium®) induced the formation of hepatocellular tumors after chronic administration to Wistar rats, but not C57BL/6 J Rj mice. Mechanistic studies in rats identified a non-genotoxic mode of action, with clear thresholds for induction of tumors, thought to involve the constitutive androstane receptor (CAR). *In vitro* studies using hepatocytes from wild type (WT) Sprague-Dawley (SD) and Wistar rat and a novel SD rat CAR knock out (KO) model (SAGE Labs, Boyertown, PA, USA) were conducted. Additional studies using human hepatocytes assessed the relevance of the rat *in vivo* results. Hepatocytes were treated with several Fluxapyroxad concentrations to evaluate induction of cytochromes (CYP) 1A, 2B, 3A and 4A using standard assays (EROD, PROD, BROD, benzyloxyquinoline-O-debenzylation, 12-hydroxylauric acid formation, Acyl CoA oxidation) and/or by Taqman® mRNA analysis. Cell proliferation was determined by nuclear incorporation of Brd-U and immunostaining. Enzyme assays indicated marked CYP2B and 3A induction in WT Wistar and SD rat hepatocytes, but only marginal increases in CYP1A and 4A. In CAR KO rat hepatocytes, there was no substantial effect on CYP2B, while induction of CYP1A, 3A and 4A was comparable to that observed in WT rat hepatocytes. Measurement of mRNA levels in WT and KO rat hepatocytes confirmed the CYP enzyme activity results. Cell proliferation was induced in WT, but absent in CAR KO rat hepatocytes. Results from human hepatocyte studies showed that Fluxapyroxad induced CYP2B6 and CYP3A4, as evidenced by enzyme and mRNA measurements, but without increasing cell proliferation. These studies confirmed the role of CAR in the induction of rat liver tumors by Fluxapyroxad. The absence of increases in cell proliferation in human hepatocytes demonstrate the lack of human relevance of rat liver tumor development by Fluxapyroxad.

2707 Evaluation of the Potential for Formaldehyde Inhalation to Cause Nervous System Effects

A. D. Kraft, G. S. Cooper and B. S. Glenn. *US EPA, Washington, DC.*

Formaldehyde is a high production volume chemical with a variety of uses to which people are exposed through work (e.g., embalmers; garment workers), from common household items such as wood furniture and stoves, and from outdoor air where it is present primarily as a product of combustion. In general, outdoor formaldehyde levels are low compared to those found in indoor settings. While formaldehyde inhalation is known to cause eye irritation and cancer in humans, the evidence for other health effects, including nervous system effects, is less clear. Our analysis systematically identifies, considers, and integrates the evidence from studies evaluating endpoints related to neurotoxicity following formaldehyde inhalation in experimental animals and humans. After identifying the studies through a systematic search and screening process, the studies were evaluated using criteria developed to assess the rigor, sensitivity, and bias of the study methods. The identified studies reported an association between formaldehyde exposure and an array of nervous system effects, including the motor neuron disease, amyotrophic lateral sclerosis, effects on motor activity and anxiety, and impaired learning and memory. However, notable methodological limitations were identified for most of the assessed studies (e.g., co-exposure to methanol when formalin was used as the test article), which introduced significant uncertainties to the interpretation of the reported observations. Thus, the results across studies were considered in the context of these limitations. The data were also evaluated in light of the biological plausibility of formaldehyde in inspired air impacting the structure and/ or function of the nervous system, as defined based on an analysis of demonstrated (i.e. formaldehyde-specific) and/ or established (e.g., from related chemicals) mechanisms. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

2708 Application of a Surrogate Analysis Approach to Identify Toxicity Surrogates for N-Heptanal, N-Heptane, and O-Aminophenol

J. Melia[1], H. Carlson-Lynch[1], M. Odin[1], J. Tunkel[1], J. Rhoades-Hamacher[1] and S. Wesselkamper[2]. *[1]SRC, Inc., North Syracuse, NY and [2]US EPA, Cincinnati, OH.*

A tiered surrogate analysis approach that relies on structure-activity relationship (SAR) principles (Wang et al., 2012; Regul. Toxicol. Pharmacol.) was applied to identify toxicity surrogates for the data-poor chemicals n-heptanal, n-heptane, and o-aminophenol. Three types of surrogates (structural, metabolic, and toxicity-like) were evaluated. Structural surrogates were assessed by performance of similarity searches in DSSTox and ChemIDplus. Search findings suggest that similarity scores can be informative but should not be solely relied upon for selecting the best surrogate compound. For example, o-aminophenol similarity scores were inconsistent for the potential analogs between ChemID and DSSTox. The ChemID and DSSTox similarity scores for n-heptanal analogs were relatively low (<50% for ChemID, 33–69% for DSSTox) due to differences in carbon chain length (analogs are C2-C5); however, chain length is not expected to affect the chemical reactivity of the aldehyde analogs. Metabolic surrogates determined via literature identified similar pathways of metabolism and common metabolites; however, absorption and distribution data may also be important. n-Nonane was eliminated as a potential surrogate for n-heptane because of species differences in vapor uptake (i.e., 10-fold higher blood:air partition coefficient in humans versus rats). A review of comparable toxicity information can prove useful in evaluating compounds as potential surrogates. For example, a 16-week inhalation study in rats exposed to n-pentane, n-hexane, or n-heptane demonstrated a decrease in mean nerve conduction velocity for n-hexane only, indicating that n-hexane is not an appropriate surrogate compound for n-heptane. These surrogate analyses provide scientifically-based predictions of toxicity and inform quantitative risk assessment of data-poor chemicals. The views expressed in this abstract are those of the authors and do not necessarily reflect the views and policies of the US EPA.

2709 Read-Across at the Crossroad of Chemoinformatics and Regulatory Science

A. Mostrag-Szlichtyng[1], I. Boyer[2], B. Bienfait[3], B. Heldreth[2], T. Kleinoder[3], J. Marusczyk[3], A. Tarkhov[3], O. Sacher[3], C. Schwab[3], V. Vitcheva[1], J. Rathman[1,4] and C. Yang[1,3]. *[1]Altamira LLC, Columbus, OH; [2]Cosmetics Ingredient Review, Washington, DC; [3]Molecular Networks GmbH, Erlangen, Germany and [4]The Ohio State University, Columbus, OH.*

Improving the predictivity of methods for human safety/risk assessment remains a key interest of regulatory programs. A diverse array of *in vivo*, *in vitro*, and computational methods are being evaluated to profile compounds for potential impact on human health. Read-across as a data gap filling technique is associated broadly with two aspects: one is the data inference of a closely related chemical structure and the other involves the synthesis of an outcome due to weight of evidence evaluation of diverse information or knowledge sources. These include analog- or category-based classifications and relevant animal toxicity, bioassays, and quantitative structure activity relationship models, results from which are combined and assessed. The read-across workflow has been captured in a decision support platform in which speciation of the target compound in terms of reactivity and metabolic pathways is determined, followed by quantifying the similarity to the target based on structural and physicochemical properties as well as the biological assay profiles. At this crossroad, chemoinformatics concepts are applied to describe analogs in terms of their reactive metabolite potential, mechanistic assays and pathway associations. These approaches rely on evaluating diverse information sources, combining data via quantitative weight-of-evidence, and ultimately providing predictions with uncertainty estimates. Examples of the read-across workflow are presented for several biosurfactants of the type that are extensively used in cosmetics as well as foods, pharmaceuticals, and agrochemicals.

2710 Evaluation of Chemically Induced Cytotoxicity of Read Across Compounds in Rat and Human Lung Tissue

O. Danov[1], S. Escher[1], K. Schröder[1], J. Koschmann[3], M. Niehof[1], D. Vorgrimmler[2], P. Braubach[4], D. Jonigk[4], T. Hansen[1], A. Braun[1] and K. Sewald[1]. [1]*Fraunhofer ITEM, Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Centre for Lung Research (DZL), Hannover, Germany;* [2]*Freiburg Center for Data Analysis and Modeling (FDM), Freiburg, Germany;* [3]*geneXplain GmbH, Wolfenbüttel, Germany and* [4]*Hannover Medical School, Hannover, Germany.* Sponsor: C. Dasenbrock.

Read across (RAX) is an alternative approach to evaluate chemicals for human risk assessment. Toxicity of untested chemical(s) should be predicted by tested compounds based on their structural and physico-chemical similarity. Three RAX-categories have been selected based on shared structural properties and similar toxicological effects in repeated dose toxicity studies and were evaluated in rat and human precision-cut lung slices (PCLS). Exposure of PCLS was performed on three days for three hours daily. The cytotoxicity was assessed by WST-1 assay in the dose-dependent manner. A concentration showing 10-20 % cytotoxicity was used for gene expression analysis. Vicinal halogenides showed decreasing cytotoxicity from 1,2-Dibromo-3-chloropropane (DBCP, IC50=0.3mM), to 1,2,3-Trichloropropane (IC50=4mM) followed by 1,2-Dichloropropane (1,2-DCP, IC50>10mM) in rat PCLS and 3.3mM, and over 10mM for both other chemicals in human PCLS, respectively. Aromatics showed decreasing cytotoxicity from 2-Naphtol, to 2-Methylnaphthalene with comparable IC50 values in both species: 0.4mM vs. 0.9mM (rat vs. human) and 0.5mM vs. 0.4mM, respectively. Naphthalene was non-toxic in human lung sections at the highest tested concentration of 1mM while showing 20 % vitality reduction in rat PCLS at the same concentration. Esters showed comparable IC50 values in both species. Differentially expressed genes (DEG) were analyzed in human tissue sections. Up- and down-regulated genes were assessed for all tested substances. The number of DEGs ranged from 16 for 1,2-DCP to over 600 DEGs for DBCP. DEGs could be mapped to biological processes such as regulation of cytokine secretion, neutrophil chemotaxis or apoptotic process. This evaluation of rat and human PCLS showed that *ex vivo* chemical testing results in a promising approach for toxicity profiling and assessment of interspecies diversity. Moreover, evaluation of chemically induced gene regulation can reveal similarities and differences within the groups. This work was supported by BMBF grant 031A269A.

2711 Toxicological Categorization of P- and E-Series Glycol Ethers Using High-Content Screening of Human Induced Pluripotent Stem Cell (iPSC)-Derived Cells

Y. Iwata[2], F. Grimm[2], M. Wilson[2], M. Bittner[2], O. Sirenko[3], J. C. Rowlands[1], N. Ball[1] and I. Rusyn[2]. [1]*The Dow Chemical Company, Midland, MI;* [2]*Engineering Experiment Station, Texas A&M University, College Station, TX and* [3]*Veterinary Integrative Biosciences, Molecular Devices, LLC, Sunnyvale, CA;* [4]*Veterinary Integrative Biosciences, Texas A&M University, College Station, TX.*

High-content screening (HCS) assays utilizing novel organotypic cell culture models are an attractive approach for predictive safety assessments of chemicals through biological data-based read across. To test the hypothesis that HCS represents a feasible approach to categorize chemicals based on similarities in their *in vitro* toxicity profiles, we screened eight propylene (P-series) and twelve ethylene (E-series) glycol ethers, structurally related yet toxicologically diverse group of prototypical industrial high production volume chemicals. We used two human induced pluripotent stem cell (iPSC)-derived cell types, cardiomyocytes and hepatocytes. Cells were exposed to glycol ethers in concentration-response over five (semi-)logs for up to 48 hours. Toxicity endpoints included effects on cardiomyocyte beating patterns. Cytotoxicity, mitochondrial and cytoskeletal integrity, and (in hepatocytes) reactive oxygen species formation and lipid accumulation were also assessed. Data were fit to a concentration-response to derive point-of-departure values that were used as quantitative descriptors for categorization in Toxicological Prioritization Index (ToxPi). We found that there is a correlation between the length of the alcohol group and induced effects such that glycol ethers can be categorized based on simple glycols, methyl-, ethyl-, propyl-, butyl-, and hexyl ethers. Within an alcohol group based category, in general there was increasing cytotoxicity between mono-, di-, and tri-substituted glycol ethers. A trend was observed with integrative ToxPi evaluation that combined all endpoints and cell-types, which was more prominent than when the data was expressed by individual cell-type. However, the trends in *in vitro* data did not align with the data trends in *in vivo* toxicity for these substances as there was no separation between E- and P- series glycol ethers in terms of biological activity. Future efforts should be directed towards correlating biological data and physico-chemical data sets and gene expression analysis as additional means to define biological similarity.

2712 Isomer-Specific Toxicity Profiles of Aminophenols

S. Perumal Kuppusamy[2] and J. Lipscomb[1]. [1]*National Center for Environmental Assessment, US EPA, Cincinnati, OH and* [2]*Risk Evaluation Branch, National Institute for Occupational Safety and Health/Centers for Disease Control and Prevention, Cincinnati, OH.*

Aminophenols are used in photographic processes, and in several fluorescent and hair dyes. Because the isomers differ in metabolism and the capabilities to cause toxicities in different organs, the repeated dose toxicity profiles for aminophenol isomers (ortho-, meta- and para-) are compared. Ortho aminophenol (OAP) is converted to 2-aminophenoxazine-3-one by cytochrome c and excreted as sulfate and glucuronide conjugates. The critical effect is increased relative liver weight in male rats at 83 mg/kg-day on 12 days oral exposure. No observed adverse effect level (NOAEL) could not be identified. For metaaminophenol (MAP), only glucuronidation and sulfation conjugation reactions are reported. The critical effect is reduced body weight, tremors, and increased serum bilirubin in newborn rats of both sexes at 240 mg/kg-day on postnatal days 4-21 oral exposure. NOAEL is 80 mg/kg-day. Paraminophenol (PAP) is oxidized to p-benzoquinoneimine by renal tissue cytochrome P-450 and binds to renal protein. PAP acetylates and conjugates with glutathione for excretion. The critical effect is brown urine, increased epithelial cells in the urine, and increased proximal basophilic tubule in the kidney of both sexes of rats at 100 mg/kg-day on 28 days oral exposure. NOAEL is 20 mg/kg-day. The severe nephrotoxic effect of PAP, among other isomers, may be due to para arrangement on the benzene ring of hydroxyl and amino groups. OAP and PAP produced dose-related increases in developmental toxicity in Syrian Golden Hamsters with intraperitoneal injection. Although both positive and negative genotoxicity results are reported for all isomers, there is inadequate information to assess the carcinogenic potential for all of them. No repeated dose inhalation studies for all aminophenols are located. Taken together, each isomer target different organs. These kind of studies improve our understanding of the different risks posed by different arrangement of atoms in the molecule. [The findings and conclusions in this presentation are those of the authors and do not necessarily represent the views of the NIOSH or the US EPA.]

2713 Multi-Dimensional Analysis of Mode of Action: Identifying the Critical Factors for Determining Risk

G. M. Woodall, A. K. Hotchkiss and A. M. Jarabek. *National Center for Environmental Assessment, US EPA, Research Triangle Park, NC.*

Human exposures to chemical pollutants are often not at uniform concentrations for constant, continuous periods of time; this represents a challenge to evaluation of risk and developing effective risk mitigation approaches based on real-world exposure scenarios. A simple, two-dimensional dose-response relationship (increasing response or severity versus increasing exposure levels) is typically used to describe toxicological data. Factors other than dose can affect an adverse outcome *in vivo*, and can be outcome- or chemical-specific, including: duration of exposure; nature of exposure (single or repeated peaks versus long-term average); recovery time between exposures; timing of exposure (time of day, life-stage, presence of other stressors); and many others. The dose-response relationship is also dependent on the mode of action (MOA) and choice of dose metric. A two-dimensional dose-response relationship is insufficient to comprehensively describe these relationships. Instead, we are using a multi-dimensional surface analysis with exposure concentration, duration, and timing as independent variables. To test this analytical approach, we reviewed the existing literature for several chemical classes (e.g., VOC, aldehyde, aromatic hydrocarbons, metals, anions) to characterize the relative contribution of each for explaining the surface response function for a spectrum of endpoints (e.g., respiratory, reproductive/developmental, liver). This type of information will be useful for making health risk decisions to mitigate risk based on the most critical factors (e.g., if repeated peak exposures are more relevant than long-term averages, an acute reference value may be more relevant than a chronic reference value). The results of this analyses are compared to existing health effect reference values, with recommendations on how best to derive relevant risk estimates for acute and episodic exposures based on chemical class and adverse outcome. (The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA).

2714 Thymomas in Fischer 344/N Rats in the National Toxicology Program Database

G. A. Willson[1], R. R. Moore[1], H. Nagai[2], R. A. Miller[1], J. F. Hardisty[1], N. Allison[1] and D. E. Malarkey[2]. [1]*Experimental Pathology Laboratories, Inc., Research Triangle Park, NC and* [2]*Cellular and Molecular Pathology Branch, National Institute of Health Sciences, Research Triangle Park, NC.* Sponsor: J. Hardisty.

Thymoma is an uncommon tumor in most strains of rats and mice. Primary tumors of the thymus of Sprague-Dawley-derived rats (Tif:RAI) were reported from 7 different long-term studies (Naylor, et al., 1988). In that study, 192 primary thymic tumors were identified (out of 4281 animals), of which 171 appeared benign and 21 appeared malignant. In the F344/N rat spontaneously occurring thymomas are rare, and most of those have been reported to be benign (Haseman, Hailey, and Morris, 1998). The literature contains no reports of large studies of thymoma in F344/N rats. The purpose of this study is to describe the morphological features, incidence, and behavior of thymoma in F344/N rats in NTP studies. Two hundred and seventy seven mediastinal masses of thymic epithelial origin were observed from the NTP database of approximately 175,000 rats from chronic studies and none were found in approximately 15,000 rats from subchronic studies. Thymomas occurred more commonly in male rats (156/277 [56.3%]) than in female rats (121/277 [43.7%]). Two hundred thirty five were considered benign and 42 malignant, 14 of which exhibited metastases. Metastasis was most frequent to the lung (14), then lymph node (1), and liver (1). Malignant thymomas showed a propensity to be the cause of death and to result in early mortality. No occurrences of this rare F344/N neoplasm appeared to be related to exposure to test articles. References: Naylor, D.C., Krinke, G.J., and Ruefenacht, H.J. (1988). Primary Tumours of the Thymus in the Rat. J. Comp. Path. Vol 99, PP 187-203. Haseman, J.K., Hailey, J.R., and Morris, R.W. (1998). Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. Toxicol Pathol 26, 428-441.

2715 Lung Tumor Incidence and Survival for CD-1 Mice: Comparison of 78 and 104 Week Studies in the Historical Data

C. M. Lewis. *Dept. of Pesticide Regulation, CalEPA, Sacramento, CA.*

In 2010 and 2012 the California Department of Pesticide Regulation completed human health risk assessments for the pesticide chloropicrin that included an evaluation of its carcinogenic potential. Chloropicrin is genotoxic *in vitro* and caused tumors in rodent oral and inhalation bioassays. A quantitative cancer analysis was performed based on an 18-month inhalation study that showed statistically elevated incidence in lung tumors in female CD-1 mice. In the weight of evidence for carcinogenicity, there was some uncertainty because the combined incidence of adenomas and carcinomas in the females (22/49 or 45%) was only marginally statistically significant, partly due to the high incidence in the control group (13/48 or 27%, all adenomas). It was conceivable that the tumor incidences at the high dose could be higher had the study duration been longer (e.g., 2 years). At the time, the only available historical control data for CD-1 mice was from studies performed between 1987 and 1996. Since then, two new reports were published covering more recent studies. The lung tumor incidences and survival rate in the 78-week and 104-week studies from these 3 historical reports (all from Charles River) were compared. In the 78-week studies, the range of pulmonary adenomas and carcinomas in female CD-1 mice was 0–16.0% and 0–12.0%, respectively; the combined incidence was 1.7–25.7%. In the 104-week studies (24 studies) the range was 0-26.7% for adenomas and 0–20.0% for carcinomas; the range for the combined incidence was 3.9–35%. The incidence of pulmonary adenomas in the control group in the chloropicrin 18-month study was clearly outside the historical range for the 78-week studies and even outside the historical range for the combined incidence. The historical control range increased with the longer studies, mostly due to increases in adenomas. The survival rate of control females ranged from 63.3–83.3% in the 78-week studies and 18.6–55.4% in the 104-week studies. While survival rate decreased from 78 week to 104 week, it was above the 25% cutoff recommended by US EPA guidelines, except for one study. Therefore, it should be possible to conduct a 2-year oncogenicity study using CD-1 mice. However, health issues at the end of life expectancy including increased incidence of tumors in the control may compromise the overall interpretation of the tumor data. Use of a different strain with greater longevity (B6C3F1 mice) may be a way to overcome survival/health issues.

2716 Evaluation of the Magnitude of the Age-Dependant Adjustment Factor (ADAF): A Kinetics-Based Case Study with Trichloroethylene and Vinyl Chloride

M. Valcke[2,3], H. Tohon[2], A. Nong[1] and S. Haddad[2]. [1]*Health Canada, Ottawa, ON, Canada;* [2]*Université de Montréal, Montreal, QC, Canada and* [3]*Direction de la santé environnementale et de la toxicologie, INSPQ, Montréal, QC, Canada.*

An age-dependant adjustment factor (ADAF) is applied in cancer risk assessment for infants aged <2 yrs (10-fold, INF) and children aged 2 –<16 yrs (3-fold, CHILD) to account for early-life susceptibilities to mutagenic carcinogens. This study presents a proof-of-concept evaluation of the magnitude of the kinetic component of ADAF (ADAFk) for chemicals that may occur simultaneously in the environment. Simplified PBPK models for vinyl chloride (VC) and trichloroethylene (TCE) were used to simulate multi-route exposure via drinking water (100 and 200 µg/L respectively) in adults (AD, 30 yrs), CHILD and INF. Ingestion, dermal contact while bathing and the 24-h inhalation of resulting vapors in the bathroom and house were considered. Binary exposure, and ternary exposure with tetrachloroethylene (500 ug/L), were also simulated assuming competitive inhibition of CYP2E1 oxidative hepatic metabolism. Monte Carlo simulations were performed in order to generate distributions of relevant internal dose metrics (amount of mutagenic metabolites produced, normalized to tissue volume), based on which the ADAFk was quantified. Precisely, the ratio of the 95th percentile value in INF or CHILD over the median in AD was calculated. Ratios were computed for the glutathione-S-transferase-mediated TCE metabolites produced in kidneys (AGSTkid) and in both kidneys and liver (AGSTkl), as well as for CYP2E1-mediated VC metabolites produced in the liver (ACYPliv). Median values in AD for AGSTkid, AGSTkl and ACYPliv for single substances were respectively 0.38, 0.52 and 0.11 mg/L.day. Corresponding ADAFk were respectively 2.37, 1.91 and 1.34 for INF and 2.69, 2.54 and 1.43 for CHILD. GST-based ADAFk slightly decreased (≈10%) in mixtures, presumably in part due to metabolic interactions. In conclusion, this case study suggests that default ADAF values adequately accounts for CHILD- and INF-specific variations based on ADAFk for TCE and VC. This is true for those chemicals alone or in the specific mixtures evaluated, and for concentrations in drinking water that are greater than what's generally encountered in the environment.

2717 An Evaluation of Transplacental Carcinogenesis for Human Health Risk Assessment

B. Foos[4], M. Mabson[2], J. Rogers[3], B. Sonawane[4] and G. Ginsberg[1]. [1]*Connecticut Department of Health, Hartford, CT;* [2]*Oak Ridge Institute for Science and Education, Washington, DC;* [3]*US EPA, Research Triangle Park, NC and* [4]*US EPA, Washington, DC.*

Risk assessments take into account the sensitivity of the postnatal period to carcinogens through the application of age-dependent adjustment factors (ADAFs) (Barton et al. 2005). The prenatal period is also recognized to be sensitive but is typically not included into risk assessments (National Research Council, 2009). An analysis by California Office of Environmental Health Hazard Assessment (2008) contrasted prenatal, postnatal and adult sensitivity to 23 different carcinogens across 37 studies. That analysis found a wide range of transplacental sensitivity with some agents nearly 100 fold more potent *in utero* than in adults while others had an *in utero*/adult ratio less than one. Our review of this database has identified nine chemicals which have data that can be used to compare cancer potency from prenatal vs postnatal exposure. All nine are genotoxic carcinogens, some requiring metabolic activation and others being direct acting. The rank order potency for these chemicals is similar across life stages with three chemicals having the greatest potency in both pre- and postnatal stages (benzidine, safrole, ethylnitrosourea, 10–100 fold greater than adult only exposure). Five carcinogens had more modest ratios to adult potency in both pre- and postnatal testing (vinyl chloride, ethylnitroso biuret, 3-methylcholanthrene, urethane, diethylnitrosamine, 3–10 fold). Only one chemical showed a pre- vs postnatal divergence (butylnitrosourea, prenatal less than adult, postnatal 6x greater than adult). Based upon this limited set of genotoxic carcinogens, it appears that the prenatal period often has a sensitivity that approximates what has been found for postnatal, and the maternal system does not offer substantial protection against transplacental carcinogenesis in most cases. This suggests that the system of ADAFs developed for postnatal exposure may be considered for prenatal exposures as well. An alternative approach may be to calculate cancer risk for the period of pregnancy rather than blend this risk into the calculation of lifetime risk. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the policy of the US Environmental Protection Agency or State of Connecticut.

2718 Interpretation of Fish Biomarker Data for Regulatory Identification, Classification and Testing of Endocrine Disrupting Chemicals

Z.-C. Dang and C. Bodar. *RIVM, Bilthoven, Netherlands.*

Chemical induced changes in fish biomarkers vitellogenin (VTG), secondary sex characteristics (SSC), and sex ratio indicate mechanisms/modes of action (MOAs) of EAS (estrogen, androgen and steroidogenesis) pathways. These biomarkers could be used for defining MOAs and the causal link between MOAs and the adverse effects in fish for the regulatory identification of endocrine disrupting chemicals (EDCs). This study compiled data sets for 172 chemicals including 149 chemicals for VTG, 57 chemicals for SSC and 38 chemicals for sex ratio in fathead minnow, medaka and zebrafish. This evaluation showed that 1) both false positives and negatives were observed for fish biomarkers; 2) chemicals with non-EAS pathways could also induce changes in fish biomarkers; 3) responses of fish biomarkers did not always follow the anticipated patterns of EAS pathways. These responses may result from the interaction of chemical-induced multiple MOAs and confounding factors like fish diet, infection, culture conditions, general toxicity and stress response. The complex response of fish biomarkers to a chemical of interest requires EDC testing at multiple biological levels. Interpretation of fish biomarker data should be combined with relevant information at different biological levels, which is critical for defining chemical specific MOAs. The relevance of fish biomarker data for the identification, classification and testing of EDCs in the regulatory context was discussed. It is emphasized to follow a weight of evidence approach for the interpretation of fish biomarker data and there is a strong need for defining data requirements for the regulatory identification of EDCs.

2719 Dosimetry for Silver Nanoparticle Exposure *In Vitro*

K. Riaz Ahmed, A. Nagy, S. Malghan, P. Goering and R. Brown. *CDRH, US FDA, Silver Spring, MD.*

Smaller sized nanoparticles are typically more potent than larger particles in *in vitro* cytotoxicity assays when the concentration associated with a toxic response is expressed on a mass per volume basis. The use of surface area per volume has been proposed as a more appropriate dose metric to express *in vitro* toxicity since the solubility and toxicity of nanoparticles may be related to their surface area rather than mass. The purpose of this study was to investigate the *in vitro* dosimetric relationships of silver nanoparticles (AgNPs) across particle sizes. To accomplish this goal, Lowest Observed Adverse Effect Concentration (LOAEC) values were identified in published studies that investigated the *in vitro* cytotoxic effects of different sized AgNPs. LOAECs for each size of AgNP used in the studies were expressed in units of mass per volume, surface area per volume, and particle number per volume. Evaluation of data across studies suggested that significant size-dependent differences in toxicity existed when LOAECs (mass per volume) were compared across particle sizes, with the smallest AgNPs being the most toxic. In contrast, larger AgNPs were more toxic when the LOAECs were expressed using a particles/ml dosimetric. Adjusting the AgNP concentrations on a surface area basis resulted in more uniform LOAECs for particles of different sizes. To complement this multi-study analysis, we assessed cell viability in L929 fibroblast cells treated with 10, 100 or 200 nm AgNPs for 24 h. Our results indicated that the 10 nm AgNPs were 25- and 50-fold more toxic to L929 cells than 100 and 200 nm AgNPs when the dose was expressed in a mass per volume basis. In contrast, 100 and 200 nm AgNPs were 40- and 160-fold more toxic than 10 nm particles when LOAECs were expressed as particles/ml. Use of a surface area-based dosimetry normalized these effects; 10 nm AgNPs exhibited only 2.5-fold increased toxicity compared to larger particles. The results of this study will help risk assessors to better extrapolate the *in vitro* cytotoxic effects of AgNPs from one particle size to another through use of a uniform surface area based dose metric. This work will also contribute to mitigating inter-assay variability and aid in the benchmarking of AgNPs for regulatory purposes.

2720 Effect of Aliskiren Nanoformulation on Rat Kidney Function

D. E. Murrell[3], H. Oakes[3], J. Coleman[2], A. V. Hanley[3], K. W. Bullins[3], G. A. Hanley[2] and S. Harirforoosh[3]. [1]*College of Arts and Sciences, East Tennessee State University, Johnson City, TN;* [2]*Division of Laboratory Animal Resources, East Tennessee State University, Johnson City, TN and* [3]*Pharmaceutical Sciences, Gatton College of Pharmacy East Tennessee State University, Johnson City, TN.*

Aliskiren, a direct renin inhibitor, is an anti-hypertensive drug presenting with low bioavailability and high drug cost. Our laboratory has recently developed an aliskiren poly(lactide-co-glycolide) nanoparticle (NP-ALS). This study compared NP-ALS versus a methylcellulose aliskiren suspension (ALS) in terms of kidney function. Blood urea nitrogen (BUN), kidney injury molecule-1 (KIM-1), creatinine, and electrolyte levels were assayed in plasma and/or urine. Baseline (B) plasma samples were taken and urine was collected (≈12 hrs) on day 0. ALS (n=7; 30 mg/kg) or NP-ALS (n=7; ALS dose equivalent) was dosed on day 1 then serial blood samples were taken and urine was collected (≈12 hrs). Final (F) plasma samples were taken 24 hrs post-dose. Data are presented as mean±standard deviation. SPSS identified outliers and the Mixed Procedure two-way ANOVA component of SAS determined significance ($p<0.05$). UN increased significantly after dosing in both groups (NP-ALS-B vs NP-ALS-F, 12.34±0.91 vs 15.14±0.70 mg/dL; $p<0.0001$ and ALS-B vs ALS-F, 13.17±0.27 vs 15.60±1.27 mg/dL; $p<0.0001$); however, no statistical difference was found between endpoint levels. KIM-1 increased following ALS dosing (ALS-B vs ALS-F, 0.11±0.03 vs 0.17±0.05 ng/mL; $p=0.0486$); while NP-ALS showed a decrease (NP-ALS-B vs NP-ALS-F, 0.13±0.05 vs 0.07±0.04 ng/mL; $p=0.027$) which was also significantly decreased compared to ALS-F ($p=0.0008$). Plasma creatinine levels were elevated following both dosage forms (NP-ALS-B vs NP-ALS-F, 0.96±0.31 vs 1.63±0.17 mg/dL; $p<0.0001$ and ALS-B vs ALS-F,0.87±0.50 vs 1.50±0.21 mg/dL; $p<0.0001$) with no significance change between endpoint values. Urinary creatinine and Na excretion rates along with plasma electrolyte levels were unchanged. Urinary K excretion was decreased significantly only in the ALS group (ALS-B vs ALS-F, 0.16±0.02 vs 0.09±0.03 µmol/min/100g; $p=0.0274$) which was also significantly reduced compared to NP-ALS-F (ALS-F vs NP-ALS-F, 0.09±0.03 vs 0.19±0.13 µmol/min/100g; $p=0.016$). At the dosage tested, NP-ALS showed favorable kidney changes compared to ALS in regard to KIM-1 and K excretion rate. Thus NP formulation may reduce ALS-induced kidney dysfunction.

2721 Increased Tubular Dysfunction and Injury Response Biomarkers in the Absence of Overt Clinical Nephrotoxicity in Cancer Patients Receiving Cisplatin Therapy

B. George[1,3], X. Wen[3], N. Johnston[2], M. Gomez[2], L. Ellison[2], M. S. Joy[2] and L. M. Aleksunes[3]. [1]*Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ;* [2]*Department of Pharmaceutical Sciences, University of Colorado, Aurora, CO and* [3]*Department of Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Novel urinary proteins have emerged as more sensitive indicators of acute kidney injury compared to serum creatinine and may provide clues regarding the mechanism and anatomic location of nephrotoxicity. We sought to characterize urinary concentrations of biomarkers that reflect an acute injury response (KIM-1, clusterin, NGAL, TFF3), glomerular and tubular dysfunction (cystatin C, calbindin, GST-π, albumin, β2MG) and inflammation (IL-18, MCP-1, osteopontin) after exposure to cisplatin. Patients (n=57) with solid tumors and scheduled for chemotherapy with cisplatin ≥ 25 mg/m2 were recruited. Urine was collected at baseline, 3, and 10 days post infusion and analyzed using a multiplex protein assay. Markers of tubular dysfunction (β2MG, cystatin C, albumin) were elevated at day 3 by 330%, 185%, and 147%, respectively. By day 10, levels of calbindin and GST-π were increased by 827% and 160%, respectively. Markers of injury and inflammation (KIM-1, clusterin, and MCP-1) were also elevated at day 10. Although reported to be down-regulated in tubular injury in rats, levels of TFF3 in cisplatin-treated patients were increased at days 3 (170%) and 10 (203%). Interestingly, serum creatinine and urinary concentrations of pro-inflammatory proteins IL-18 and osteopontin were not altered. ROC curves calculated from pre- and post-cisplatin infusion concentrations revealed that β2MG and TFF3 had the highest AUC for day 3 (0.79 and 0.68, $p<0.002$), while calbindin, TFF3, KIM-1, and MCP-1 AUCs (0.76, 0.69, 0.68 and 0.68, $p<0.003$) were highest at day 10. These data suggest that novel urinary protein biomarkers may reveal mechanisms and time-dependent subclinical kidney injury in the absence of clinically detectable damage in patients receiving cisplatin. Supported by an AFPE Fellowship, DK080744, DK093903, and CA072720.

2722 Correlating Molecular Signatures and Histopathology to Identify Region Specific Lesions in Sub-Acute Drug Induced Kidney Injury

S. Dremier[1,3], P. De Ron[3], S. Snelling[2,3], P. Winlow[2,3], S. Glineur[3] and A. Nogueira da Costa[3]. [1]*Euroscreen SA, Gosselies, Belgium;* [2]*Faculty of Biological Sciences, Leeds, United Kingdom and* [3]*UCB Biopharma SPRL, Braine L'Alleud, Belgium.* Sponsor: J.-P. Valentin.

Drug attrition due to drug-induced kidney injury (DIKI) is a cause of drug failure in pre-clinical studies. Although FDA qualified pre-clinical urinary DIKI biomarkers allow for early detection of acute nephrotoxicity, increased kidney lesion specificity is still warranted. This work aims at correlating histopathological findings with molecular endpoints to characterize region-specific nephrotoxicity. A 4-week toxicity study was conducted in Wistar rats. Nephrotoxicants were selected from the literature and administered individually to independent groups (n=24) - cisplatin (tubules, 2.5mg/kg single intraperitoneal (i.p.)), puromycin (glomerulus, 20mg/kg/d i.p.) and N-phenylanthranylic acid (NPAA) (collection duct, 500mg/kg/d orally). Samples were collected prior to compound administration, at days 2, 4, 8, 15, 22 and 29. Histopathology findings confirmed region specificity of each administered compound. As from day 8, cisplatin-induced lesions consisted of minimal to moderate tubular degeneration/necrosis; puromycin induced an increase in glomerular vacuolation; and NPAA induced renal papillary degeneration. In cisplatin treated animals, KIM-1 levels showed a 15-fold increase by day 4; in puromycin treated animals, cystatin C levels increased up to 20-fold from day 15; and in NPAA treated animals, a-GST levels decreased up to 10-fold from day 2. Toxicogenomic analysis in a time-course manner identified compound-specific changes in mRNA expression profiles. Dysregulation in ion transport and cell adhesion were attributed to cisplatin-induced lesions; dysregulation in apoptotic and tissue regeneration events were identified in puromycin-induced lesions; and necrotic, inflammatory and xenobiotic metabolic processes were associated with NPAA. A panel of 68 urinary miRNAs associated with kidney injury, including miR-193, miR-10b, miR-132 and miR-183, are currently being assessed aiming at increasing the robustness of region-specific molecular biomarker signatures. On-going integration efforts will further reinforce these findings with the objective of characterizing region-specific DIKI. Overall, our findings suggest potential tubular, glomerular and collection duct specific molecular signatures of a sub-acute nature.

2723 Evaluation of Kidney Biomarker Luminex Assay Panels Using a Rat Cisplatin Model

T. Erkens, M. Byloos, N. Goeminne, A. Kegels, L. Lammens and P. Vinken. *Preclinical Development & Safety, Janssen R&D, a division of Janssen Pharmaceutica NV, Beerse, Belgium.* Sponsor: W. Coussement.

Seven urinary kidney biomarkers were accepted as qualified by FDA and EMEA for the detection of acute drug-induced nephrotoxicity in rats in non-clinical drug development. Because of the importance of early detection of renal damage, the objective of this study was to evaluate the analytical performance of 3 Bio-Plex® Luminex assay panels using urine samples with baseline and increased levels of renal biomarkers. In addition, results were compared to a previous cisplatin study which used other Elisa and multiplex assays, and basal levels were compared to published data. Cisplatin, a known nephrotoxic agent, was administered once daily intraperitoneally at 1 mg/kg body weight for 5 days to 10 male Sprague-Dawley rats. Urine samples were collected twice prior to start of the study to establish baseline values and after 3 and 5 days of cisplatin administration. The Bio-Plex Pro RBM Rat Kidney Toxicity Panel 1 (clusterin, IL-18, MCP-1, KIM-1, osteopontin), Panel 2 (B2M, calbindin, cystatin-C, NGAL), and Albumin Kit were used on a Luminex Magpix instrument. A 5-PL curve fitting algorithm could be used for most parameters. Intra- and inter-assay precision were considered acceptable for all parameters (CV <30%), except IL-18. Parallelism was observed for all parameters except for osteopontin which showed increased concentrations with increasing dilution. The minimum required dilution varied with parameter. Selectivity met requirements for most parameters (CV <22%). The analytical performance of the Bio-Plex® assays was considered acceptable for most parameters and they were able to detect urinary increases in albumin, clusterin, KIM-1, B2M and cystatin-C (part of the FDA/EMEA qualified panel), and in MCP-1 and NGAL. Although absolute concentrations sometimes differed, for most parameters Bio-Plex® results were fairly comparable to a previous cisplatin study in which other Elisa and multiplex assays were used, and basal concentrations fell within at least one of the published reference ranges.

2724 Predictive Biomarkers for Screening the Drug-Induced Nephrotoxicity Using Co-Culture System

E.-H. Lee[1], S.-M. Park[1], M.-S. Dong[2], S. Yoon[1] and J.-H. Oh[1]. [1]*Department of Predictive Toxicology, Korea Institute of Toxicology, Daejeon, Korea, Republic of and* [2]*School of Life Sciences and Biotechnology, Korea University, Seoul, Korea, Republic of.* Sponsor: J.-H. Oh.

There have been intensive efforts to identify the nephrotoxic biomarkers for preclinical and clinical use and several representative molecules including KIM-1 and clusterin has been suggested as the *in vivo* nephrotoxic biomarkers. However, application of *in vivo* biomarkers are limited to screen the potential nephrotoxic chemicals because of low accuracy under *in vitro* system. Using *in vitro* kidney cells, the effects of metabolic activation under liver could not be reflected in the singly kidney cell culture system. Here, we developed the co-culture platforms including liver, kidney and endothelial cell culture to reflect the metabolic activation under liver and interaction with endothelial cells. We established the CYP overexpressed HepG2 cell lines (HepG2/CYPs) which stably co-express the major CYPs enzymes, CYP3A4, CYP1A2, CYP2B6, and CYP2E1. Using HepG2/CYPs, HK-2, and HUVEC, we optimized the co-culture assay system for evaluating the nephrotoxicity. The *in vitro* potential nephrotoxic biomarkers are identified by genomic analysis of HepG2/CYP, HK-2, and HUVEC cells after exposure of 10 different nephrotoxic chemicals. This data may contribute in elucidation of the molecular events during *in vitro* kidney cells after activation of hepatic metabolism and to discover the predictive biomarkers for screening the drug-induced nephrotoxicity. This research was supported by a grant (13182MFDS988) from Ministry of Food and Drug Safety in 2015, Republic of Korea. Keywords : Biomarkers, nephrotoxicity, co-culture system, gene expression profiling

2725 Serum Levels of the Organochlorine (OC) Compound DDE and Its Possible Association with Type 2 Diabetes (T2D) in Mississippians

E. C. Meek, J. A. Crow, L. H. Mangum, M. K. Ross, R. W. Wills and J. E. Chambers. *Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.*

The heavy usage of the persistent organochlorine (OC) insecticide, DDT, during the 1950s and 1960s resulted in environmental contamination of the bioaccumulative metabolite DDE worldwide. Multiple studies have reported an association with higher OC levels, including DDE, in people with T2D compared to non-diabetics. Exposure to these persistent OCs are predicted to be higher in areas of increased agriculture where these compounds were heavily used, such as the Mississippi Delta region. The Mississippi Delta is a region of 18 rural counties that accounts for the majority of the agriculture in Mississippi. Soil samples were randomly selected from 60 sites in the Delta region and 60 sites in a non-Delta region and analyzed by gas chromatography/mass spectrometry (GC/MS) for OC residues. 80% of the Delta soil samples had quantifiable levels of DDE compared to 23% of the non-Delta soil samples and were 10-fold higher. Additionally, serum samples were randomly collected from consented patients (300) from both the Delta (150) and non-Delta (150) regions at the Veterans' Administration Hospital in Jackson, MS. Subjects selected for participation were males at least 45 years of age and were either Caucasian (C) or African American (AA). Serum samples and de-identified clinical and demographic data were collected from each subject. Serum samples were analyzed by GC/MS for OC residues including DDE. Serum DDE levels were adjusted based on serum lipid levels. Diabetics had only slightly higher levels than non-diabetics, 1251 and 1231 ng/g lipid, respectively; however, subjects from the Delta region had about 1.5-fold higher DDE levels than non-Delta subjects, 1466 and 997 ng/g lipid, respectively. AA subjects had 2.1-fold higher DDE levels than C subjects, 1713 and 803 ng/g lipid, respectively. These results suggest that higher environmental residue levels of OC compounds, such as DDE, may be important for developing a biomarker of T2D. (Supported by US EPA STAR RD-83479501-0).

2726 Metabolomic Evaluation of Methionine and Choline Deficient Diet Induced NASH in Rat Reveals Altered Hepatic NAD Biosynthesis as a Possible Contributor to Disease Progression

D. M. Nelson[2], S. Stryker[2], S.-J. Chen[2], P. Shipkova[2], M. Reily[2], V. Bhaskaran[2], U. Hanumegowda[2], E. Janovitz[2] and L. D. Lehman-McKeeman[2]. *[1]Bioanalytical and Discovery Analytical Sciences, Bristol-Myers Squibb, Princeton, NJ and [2]Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ.*

Nonalcoholic steatohepatitis (NASH) is a form of fatty liver disease characterized by severe steatosis and inflammation that can progress to fibrosis, cirrhosis and hepatocellular carcinoma. In order to identify predictive biomarkers of NASH, serum samples collected at regular intervals from rats fed a methionine and choline deficient (MCD) diet to induce NASH were subjected to metabolomic evaluation. Compared to control rats fed either standard chow (Chow) or a methionine and choline sufficient (MCS) formulated diet, MCD rats displayed statistically significant changes for numerous serum metabolites. Many of the metabolite changes detected in MCD rats after one week of the diet failed to change further over time, suggesting they likely reflected the reduced food consumption and body weight noted in MCD rats, but were likely unrelated to NASH progression. In contrast, progressive changes were noted for a smaller subset of metabolites in MCD rats. Serum levels of N-methylnicotinamide, a metabolite of nicotinamide adenine dinucleotide (NAD), increased progressively in MCD rats, reaching 3.3× control levels by eight weeks. Hepatic NAD is primarily produced by *de novo* synthesis via the tryptophan catabolism pathway and to a lesser extent through scavenger pathways. Given the change in N-methylnicotinamide, the metabolomic data were queried for changes to additional products of tryptophan catabolism. In contrast, serum levels of the tryptophan catabolites kynurenine and kynurenic acid decreased in a time-dependent manner in MCD rats. Similar diminutions were noted for multiple tryptophan catabolites in hepatic extracts, with NAD and NADP levels reaching 0.06× and 0.12× control levels, respectively, by eight weeks. NAD is a coenzyme required for various redox reactions including the catabolism of fat, suggesting the marked hepatic steatosis present in MCD rats might occur in part through deficiency of NAD. Altered homeostasis of the tryptophan-NAD pathway is a new finding in methionine and choline deficiency and the molecular basis for these changes is under investigation.

2727 Circulating Paracetamol Metabolites Accurately Predict Hepatotoxicity and Represent a New Clinical Toxicokinetic Biomarker Class

A. D. Vliegenthart[1], R. Kimmitt[1], J. Seymour[1], N. Z. Homer[1], M. Eddleston[1], A. Gray[1], D. J. Antoine[2], D. J. Webb[1], S. C. Lewis[1], D. N. Bateman[1] and J. W. Dear[1]. *[1]University of Edinburgh, Edinburgh, United Kingdom and [2]University of Liverpool, Liverpool, United Kingdom.*

The primary objective of this study was to explore the toxicokinetics of circulating paracetamol metabolites in patient samples with and without acute liver injury (ALI) from the recently published SNAP trial [1]. The secondary objective was to explore why significantly more patients treated with the antiemetic drug ondansetron developed ALI after paracetamol overdose. Paracetamol, paracetamol-glucuronide, paracetamol-sulphate (Para-Sul), paracetamol-GSH (P450 mediated), paracetamol-mercapturate (P450 mediated) and Paracetamol-cysteine (Para-Cys) (P450 mediated) were measured in plasma samples from 116 patients before, after 12 hours and at the end of acetylcysteine (NAC) treatment following a single paracetamol overdose. Patients who developed ALI had a longer paracetamol plasma half-life and a higher relative amount of metabolites formed by P450 activity. There was no difference between ondansetron and placebo treated patients. At pre-treatment the fraction of metabolites formed by the P450 pathway was significantly higher in patients that subsequently developed ALI (2.2% (1.0-43.6 vs 1.0% (0.6-1.6) P=0.0004). Receiver operator curve (ROC) analysis demonstrated that the most accurate predictor of ALI was Para-Cys/Para-Sul, with an AUC of 0.91 (0.83-0.98). By contrast, the current standard markers, alanine transaminase (ALT) and plasma paracetamol concentration had ROC-AUC of only 0.67 (0.50-0.84) and 0.50 (0.33-0.67), respectively. Post hoc analysis of the SNAP trial revealed that when pre-treatment Para-Cys/Para-Sul was added to the randomisation process, the higher incidence of ALI in the ondansetron pre-treated patients disappeared while the primary outcome was unaffected indicating that by chance more patients with a toxic metabolite profile at randomisation got ondansetron compared with placebo. In conclusion, circulating paracetamol metabolites are toxicokinetic biomarkers for accurately stratifying patients at first presentation to hospital after paracetamol overdose. [1] Bateman DN, et al. Reduction of adverse effects from intravenous acetylcysteine treatment for paracetamol poisoning: a randomised controlled trial. Lancet 2014; 383: 697-704.

2728 Fibrosis-Associated Protein and Gene Expression Patterns after Oral Hepatotoxicant Administration

B. C. McDyre[2], M. G. Permenter[1], W. E. Dennis[3] and D. L. Ippolito[3]. *[1]Excet, Inc., Fort Detrick, MD; [2]ORISE, Fort Detrick, MD and [3]US Army Center for Environmental Health Research, Fort Detrick, MD.* Sponsor: J. Lewis.

Liver damage resulting from toxic industrial chemical exposure is an occupational risk for civilian workers and the military alike. Here we examine gene and protein expression patterns in blood and tissue from rats exposed to hepatotoxicants for biomarkers of liver injury. Rats were orally exposed for 1 or 5 days to bromobenzene, carbon tetrachloride, allyl alcohol, or 4,4′-methylenedianiline (MDA). Livers were analyzed histologically and gene expression was measured by microarray. Proteins from tissue and plasma were quantified by iTRAQ and quantitative LC-MS/MS. Allyl alcohol, carbon tetrachloride, and MDA administration induced a fibrotic phenotype after 5 days. High doses of allyl alcohol, MDA, and bromobenzene induced inflammation on both days, while necrosis was evident after all exposures. Fifty-one, 27, and 24 genes were differentially expressed in animals with evidence of fibrosis, inflammation, or necrosis, respectively. Twenty-four of those genes were specific for fibrosis, 3 were specific to inflammation, and 1 was necrosis-specific. Twenty-eight and 6 proteins corresponding to microarray identified genes were differentially expressed in the tissue and plasma, respectively. In MDA exposed rats, 9 proteins were differentially expressed in both matrices. Five and 9 proteins were identified as inflammation specific in plasma and tissue, respectively. In plasma and tissue, KRT18 was upregulated and HPX was modulated. Both proteins are modulated in fibrosis. Eight genes, including Pkm, Lbp, Tagln, Cxcl16, Igfbp3, Lgals3, S100a6, were modulated in all 3 histopathologies. Comparative analysis of early (1 day) changes in protein and gene expression can predict candidate early biomarkers for toxic liver injury. Disclaimer: This research was supported in part by an appointment to the Postgraduate Research Participation Program at the USACEHR administered by ORISE through an interagency agreement between the US DOE and USAMRMC. Research was conducted in compliance with the Animal Welfare Act and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.

2729 Genetic Determinants of Metabolism and Liver Weight Gain in a Multi-Strain Panel of Inbred Mice Exposed to Trichloroethylene for Four Weeks

Y.-A. Ahn[1], S. Lee[1], H. Park[1], O. Kosyk[2], D. Kim[1], I. Rusyn[3] and S. Kim[1]. *[1]Environmental Health Sciences, Seoul National University, Seoul, Korea, Republic of; [2]Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC and [3]Veterinary Integrative Biosciences, Texas A&M University, College Station, TX.* Sponsor: S. Kim.

Trichloroethylene (TCE) is classified as a human carcinogen but there are knowledge gaps on genetic roles in the toxic mechanism. The aim of the present study is to identify the genetic determinants and their roles in liver toxicity after subacute exposure to TCE using a multi- panel of mice showing genetic diversity by strain. TCE was administered with oral gavage at 0, 10, 100, and 1000 mg/kg for 4 weeks to seven strains: 129S1/SvImJ, A/J, BALB/c, C3H/He, C57BL/6J, DBA/2 and B6C3F1. Trichloroacetic acid (TCA), a metabolite of TCE, and alanine aminotransferase (ALT) were measured in serum as an exposure/metabolism marker and a early-biological effect marker of liver toxicity. Also, physiological changes including liver weights and histopathological examination for necrosis and cell proliferation (PCNA-LI) were observed at 24-hr after last oral exposure. Liver transcriptomic data was obtained from five strains of mice exposed to TCE at the highest level and control group with Affymetrix Mouse Gene 2.1-array plate followed by conventional analysis with GeneSpring. TCE treatment resulted in dose- and strain-dependent increases in serum TCA and relative liver weight ($p<0.01$), but no evidence of ALT increase and necrosis. PCNA-LI were increased in 129S1/SvImJ exposed to TCE 1000 mg/kg ($p<0.05$). Up- and down-regulation of genes associated with peroxisome proliferator activated receptor (PPAR)-related signal transduction, fatty acid metabolism and

P450 metabolism were found by strain, which were correlated with liver weight gains ($p<0.05$). In conclusion, we confirmed the strain-dependent changes in liver weight and metabolism after 4-week exposure to TCE, which seemed associated with some genetic regulation of lipid metabolism and xenobiotic stimulus for treatment effects.

2730 Metabolome-Wide Association Study of Polychlorinated Biphenyls in Humans

S. Banton[3], P. Cirrilo[4], D. I. Walker[3], V. Tran[3], W. Hu[3], K. D. Pennell[2], D. P. Jones[3], B. Cohn[4] and S. Li[3]. *[1]Center for Neurodegenerative Diseases, Dept. of Neurology, Emory University, Atlanta, GA; [2]Dept. of Civil and Environmental Engineering, Tufts University, Medford, MA; [3]Division of Pulmonary, Allergy and Critical Care Medicine, Emory University, Atlanta, GA and [4]The Public Health Institute, Center for Research on Women and Children's Health, Berkeley, CA.*

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants linked to numerous adverse health outcomes. While effects of high-dose PCB exposure have been well characterized, the toxic mechanisms contributing to distal health outcomes from chronic, ambient exposure is not well understood. To provide insight into metabolic perturbations representing a biological response to internal PCB exposure, we performed a metabolome wide association study (MWAS) of environmentally relevant PCB congeners. High-resolution metabolomic profiling of plasma obtained from two independent cohorts (397 subjects from California and 74 subjects from Georgia, USA) was completed in tandem with congener specific quantification of PCBs by gas chromatography-mass spectrometry. We combined linear regression models with Mummichog, a software program designed for untargeted metabolomics, to test significant metabolic pathways associated with the plasma PCB levels. MWAS with congeners 138, 153, 170 and 180 identified significant pathways related to lipid and fatty acid metabolism, including carnitine shuttle, *de novo* fatty acid biosynthesis, fatty acid activation, glycerophospholipid metabolism and glycosphingolipid metabolism. These results were consistent in both study cohorts, despite their difference in collection period and geological locations. Our study thus provides strong evidence of the interaction of PCBs with specific fatty acid and lipid pathways in human populations.

2731 Drug-Induced Elevations of Plasma Bile Acids in Rats Not Associated with BSEP Inhibition

Y. Li[2], H. Duong[2], M. J. Hafey[1], R. Evers[1], K. Vlasakova[2], A. Galijatovic-Idrizbegovic[2], F. D. Sistare[2] and W. E. Glaab[2]. *[1]PPDM, Merck, Kenilworth, NJ and [2]Safety Assessment, Merck, West Point, PA.*

Drug induced liver injury (DILI) is a common toxicity observed in drug development programs and can lead to withdrawal of approved drugs from the market. Many investigations have been conducted to better understand numerous mechanisms of DILI. Recent efforts have focused on transporter inhibition, specifically liver canalicular bile salt export pump (BSEP) as one mechanism of DILI, and on the potential to use bile salts in plasma as monitorable mechanism-based biomarkers of BSEP inhibition and therefore DILI risk by this mechanism. When rats are treated with drugs that inhibit BSEP, bile acids can accumulate in hepatocytes, leading to their export into blood by transporters such as Mrp3 and Mrp4, and increases of bile acid levels in plasma. Alternatively, increases of plasma bile acid levels can result from hepatocyte necrosis or inhibition of transporter mediated bile acid uptake from plasma. To further explore such alternative mechanisms of bile acid increase in plasma, several drugs known not to be BSEP inhibitors were evaluated in rat studies. Surprisingly, several antibiotics determined not to be BSEP inhibitors demonstrated significant 2 to 13 fold increases of plasma bile acids. These results suggest an alternative mechanism involving attenuation of bacterial deconjugation of bile acids following reduction of gut microflora, and possibly some contribution from inhibition of uptake of bile salts into hepatocytes. The results of these studies further demonstrate that there are several benign mechanisms that can lead to bile acid increase in plasma, emphasizing the need for careful consideration when observing and interpreting increases of plasma bile acids.

2732 Thymic Stromal Lymphopoietin Induced by Protein and Chemical Allergens in Cultured Human Keratinocytes: A Potential Biomarker for Immediate-Type Hypersensitivity

Y. Kuroda[1,2], T. Yuki[2], Y. Takahashi[2], H. Sakaguchi[2] and H. Itagaki[1]. *[1]Faculty of Engineering, Yokohama National University, Yokohama, Japan and [2]Safety Science Reserach Laboratories, Kao Corporation, Tochigi, Japan.*

Epicutaneous sensitization of protein and chemical allergens leads to immediate-type hypersensitivity due to IgE antibodies. In addition, it has been reported that the induction of thymic stromal lymphopoietin (TSLP) in the skin is important for type 2 helper T cells and IgE production. In this study, we examined the relationship between allergen exposure and change in TSLP gene and protein expression in normal human keratinocytes (NHEKs). Protein and chemical allergens as well as human serum albumin were added to the cultured NHEKs, and TSLP mRNA or protein expression levels were measured by real-time polymerase chain reaction or enzyme-linked immunosorbent assay. The influence of cytokines [interleukin (IL)-4, IL-13, and tumor necrosis factor-alpha (TNF-α)] that mimic the atopic environment was also examined. In addition, the mRNA expression of two isoforms of TSLP (long and short) was analyzed. Wheat gluten, ovalbumin (OVA), trypsin, house dust mite extract, penicillin G, and chlorhexidine digluconate–known causative allergens inducing immediate-type hypersensitivity–demonstrated significantly enhanced TSLP mRNA expression. Moreover, TSLP mRNA expression was further increased by cytokine stimuli (IL-4, IL-13, and TNF-α). Protein allergens increased mRNA expression of long and short TSLP mRNA, whereas human serum albumin increased mRNA expression of only short TSLP. TSLP protein was found to be induced by OVA. Furthermore, TSLP release was higher with OVA in the presence of cytokines than with OVA in the absence of cytokines and was detected by wheat gluten in the presence of cytokines. However TSLP was not detected by exposure of human serum albumin with or without cytokine stimulus. We suggest that the studied protein and chemical allergens induce TSLP in NHEKs and that TSLP can be an effective biomarker for detecting immediate-type hypersensitivity.

2733 Adipocykine and Liver Injury Biomarker Assessment in Electronic Waste Recyclers

K. C. Falkner[3], L. Birnbaum[1], A. Schecter[3], H. Clair[3] and M. Cave[2,3]. *[1]National Cancer Institute, Research Triangle Park, NC; [2]Robley Rex Veterans Affairs Medical Center, Louisville, KY and [3]University of Louisville, Louisville, KY.*

Purpose: Electronic waste (e-waste) recyclers have unique exposures and may be at risk for exposure-related diseases. Recently, exposures were characterized in a cohort of female e-waste recyclers from Vietnam. Polybrominated diphenyl ether (PBDE) and lead exposures were higher than Vietnamese controls, while arsenic and lead were higher in both Vietnamese groups than in age- and gender-matched subjects from the National Health and Nutrition Examination Survey. Because PBDEs, arsenic, and lead are metabolic disruptors associated with liver injury, adipocytokines and liver injury biomarkers were measured. Methods: In archived fasting serum samples from 40 e-waste recyclers and 20 controls, the following analytes were measured: cytokeratin 18 (CK18) M30 (liver apoptosis biomarker), CK18 M65 (liver necrosis biomarker), IL-1β, IL-6, IL-8, MCP-1, TNFα, leptin, adiponectin, resistin, insulin, and PAI-1. Group means were compared by t-test. IRB approval was obtained for this study. Results: Compared to published reference ranges and laboratory cutoff values, mean fasting insulin was increased in both e-waste workers and controls (233.8±189.7 and 147.0±93.6 pmol/L) while adiponectin was decreased in both groups (17.9±9.2 and 19.0±7.0 ng/mL) Likewise, the hepatocellular necrosis biomarker, CK18 M65, was elevated in 75% of e-waste recyclers and 90% of controls. No significant differences were detected between groups. Pro-inflammatory cytokines were not elevated in either group. Conclusions: Both e-waste recyclers and matched Vietnamese controls had serologic biomarkers compatible with insulin resistance and liver injury consistent with known exposures to metabolic disruptors. While no differences were seen between groups, this could have been due to the high arsenic and lead exposures present in both groups. Chronic hepatitis B infection, prevalent in Vietnam, is another potential confounder to be evaluated. More research is needed to investigate possible insulin resistance and liver injury in e-waste workers as well as in arsenic-exposed populations in general.

2734 Investigating White Matter Hyperintensities in Pilots Using a Rat Hypobaric Exposure Model

M. Meade[3], A. Hoffmann[1,3], J. M. Gearhart[1,3], N. Reo[2] and D. A. Mahle[3]. [1]*Henry M Jackson Foundation, Wright Patterson AFB, OH;* [2]*Wright State University, Dayton, OH and* [3]*711 HPW/RHDJ, US Air Force, Wright Patterson AFB, OH.*

U-2 pilots and altitude chamber technicians show increased incidence of leukoraiosis or white matter hyperintensities (WMH) in T2 weighted magnetic resonance imaging (MRI), indicative of hypodensities linked to neuronal loss, demyelination and gliotic scarring. It has been hypothesized that exposure to extreme hypobaria, excess oxygen, or a combination of both stressors, irrespective of decompression sickness, is the primary causative factor for these WMH. However, the exact etiology and underlying mechanisms are unknown. Quantitative proteomics and metabonomics have the ability to profile a large number of proteins and metabolites, respectively, in a single analysis to determine what molecules are changed relative to a control population. Sprague-Dawley rats were exposed to 20,000 ft altitude with a control group exposed to ambient pressure and thalamus and cerebellum were removed from each. The extracted proteins were separated and analyzed with an UPLC coupled to an Orbitrap MS. Following acquisition, modulated pathways were identified with Cytoscape Functional Interaction analysis software. Preliminary data from thalamus of rats exposed to 20,000 ft showed a 6% up regulation , as well as, down regulation of 6% of proteins. Modulated proteins were involved with DNA damage repair, calcium signaling, lipid metabolism and microtubule organization. The thalamus also showed down-regulated calcium-dependent blood vessel homeostasis. The cerebellum showed less modulation with 3.5% and 4% of up and down regulated proteins with changes in response to oxygen pathways and lipid transport. Both brain regions showed up-regulation in the glutamate transporter SLC17A6 and down-regulation of Myelin Basic protein compared with controls. From these results, it is postulated that WMH may be caused by hypobaric exposure or oxygen toxicity.

2735 Influence of Selected Trace Metals on Plasma FSH, LH, E2 and Prolacting Levels in Women with Secondary Infertility Attending University College Hospital, Nigeria

I. O. Omotosho and O. A. Akinade. *Chemical pathology, University of Ibadan, Ibadan, Nigeria.*

Infertility is a universal reproductive health challenge across all races of the world; various causes including dietary intake and exposure to trace metals have been implicated. This study focused on investigating association between levels of some trace metals and reproductive hormones in clinically diagnosed secondary infertile women. The levels of the trace metals; zinc (Zn), iron (Fe), cadmium(Cd) and lead (Pb) with the reproductive hormones; luteinizing hormone (LH), follicle stimulating hormone (FSH), oestrogen (E2) and prolactin (PRL) were determined in 20 secondary infertile women (test) and 18 apparently fertile women (control) using standard laboratory methods. Median hormonal results with their inter quartile ranges were: 5.3IU/L (3.9 - 6.4IU/L), 7.4IU/L (4.5 - 14.7IU/L), 0.6nmol/L (0.3 - 0.9nmol/L) and 386.2mIU/L (193.7-524.8mIU/L) in test cases and 5.9IU/L (5.1-7.4IU/L), 7.5IU/L (5.2-15.2IU/L), 0.8nmol/L (0.3-1.2nmol/L) and 220mIU/L (177.7-292.3mIU/L) in controls for FSH, LH, E2 and PRL respectively. Median and inter quartile range for the trace metals were16.4µmol/L (11.8 - 41.2µmol/L) and 42.5µmol/L (26.3 - 59.0µmol/L) in test cases and 11.7µmol/L (7.0 - 19.3) and 13.7µmol/L(11.5 - 15.9)in controls for Fe and Zn respectively. The median and inter quartile range of blood Cd in test and control cases were 0.0007nmol/L (0.0005 - 0.0009) and 0.0007nmol/L (0.0006 - 0.0009) respectively while blood Pb was not detectable in both groups. Although non-significant differences($p>0.05$) and no correlation were observed amongst these results, a significantly higher plasma Zn level was obtained in controls (42.5µmol/L) relative to the test cases (16.4µmol/L) ($p<0.05$). Results from this study suggested normal hypothalamic pituitary gonadal axis (HPGA) in these secondary infertile premenopausal women. However, the low level of plasma zinc may be implicated as the basis of infertility in these women.

2736 Skeletal Mn Across the Lifespan: Target and Biomarker

C. Richardson[4], S. Peter[3], J. Pacheco[2], S. Fendorf[2], R. Ritchie[1], N. Dave[1], R. Lucchini[3] and D. Smith[4]. [1]*LBNL, Berkeley, CA;* [2]*Stanford, Palo Alto, CA;* [3]*University of Brescia, Brescia, Italy and* [4]*University of California, Santa Cruz, CA.*

Elevated manganese (Mn) exposure may result in accumulation of bone Mn because it can serve as a Ca analog; if so, this may provide utility as a biomarker of cumulative exposure over the lifespan. Here we determined 1) the impact of early postnatal versus lifelong Mn exposure on skeletal Mn accumulation, and the relationship with blood and brain Mn across the lifespan in rats, 2) whether elevated Mn alters bone mineral composition and skeletal strength, and 3) skeletal Mn levels in aged humans living in the vicinity of historic ferromanganese alloy plant activity. Male Long-Evans rats were orally exposed to 0, 25 or 50 mg Mn/kg/d over early life (PND 1-21) or lifelong, and sacrificed at PND 24, 66, or ~500. Femoral head samples were obtained from aged subjects (n=47) during arthroplasty. Tissue Mn levels were determined by ICP-MS, bone mineral composition in rats was determined by XRD and XAS, and rat femurs were tested for fracture resistance by Instron. Results show 1) Bone Mn levels in rats are more responsive to the concurrent oral exposure dose than blood or brain Mn levels; 2) In adolescent rats, bone Mn levels remain elevated and decline more slowly than blood Mn, and elevated bone Mn burdens from early postnatal life exposure appear to contribute to blood Mn levels; 3) There is no evidence that Mn accumulates in bone with age and lifelong oral exposure. In fact, bone, brain, and blood Mn levels decrease with age, even with ongoing elevated exposure, with age being more influential. 4) Instron analyses suggests that elevated early life Mn exposure may increase bone stiffness and reduce overall bone strength in rats, though XRD analyses do not indicate measurable alteration in the macroscopic composition of bone mineral. And 5) in aged humans, bone Mn levels averaged 0.05 ug/g (men) and 0.03 ug/g (women), with trending effects of age and sex on bone Mn levels (lower in females and older subjects). In conclusion, bone is a sensitive marker of concurrent exposure, but Mn does not accumulate in bone as does lead, but rather declines with age. Highly elevated early life Mn may reduce overall bone strength in adolescence.

2737 *In Vitro* Bioassays and Chemical Analysis of Drinking Water from Source to Tap

J. Lundqvist[2], R. Tröger[2], T. Le Godec[2], s. Ohlsson-Andersson[2], A. K. Rosenmai[2], L. Ahrens[2], K. Wiberg[2] and A. Oskarsson[2]. [1]*Dept of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden and* [2]*Dept of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Drinking water contains a complex mixture of known and unknown chemicals, where the individual compounds may be present in concentrations too low to cause effects, but added together may result in combination effects. The objective of this study was to evaluate the influence of different steps of water treatment on biological activities and chemical concentrations by using cell-based bioassays and chemical analysis. Water was sampled from five sites before the intake (including up- and downstream a waste water treatment plant (WWTP), after a sand filter, after a granulated active carbon filter, at the outlet of the drinking water treatment plant (DWTP), and from a nanofilter pilot plant. The samples were preconcentrated by solid phase extraction with two different adsorbents, HLB and ENV. Bioassays covered cytotoxicity, estrogenic activity, arylhydrocarbon receptor (AhR) activity and oxidative stress (Nrf2 activity). Chemical analysis was performed by LC/TOF-MS for 124 target chemicals, mainly medical drugs, pesticides and household chemicals. In almost all the water samples, concentrated 50 times, estrogenic, AhR and Nrf2 activities were detected. Raw water samples had in general higher activities than samples taken inside or after the DWTP, but there was no evident decrease in activity from a specific treatment step. No cytotoxic effects were revealed in any of the water samples. The total concentration of target chemicals followed another pattern and decreased considerably from downstream the WWTP to the inlet of DWTP. No biological effects were detected in a sample spiked with all the target chemicals, indicating that the activities in the water samples were not caused by the target chemicals. The study demonstrates that bioassays are valuable tools to monitor water quality and evaluate effects of water treatment.

2738 Effect of Aneugens on Primary Ciliogenesis

K. V. Divi, M. C. Poirier and O. A. Olivero. *NIH, NCI, Bethesda, MD.*

A primary cilium arises from the centrosome, and serves as a sensory organelle that communicates stimuli from the environment to the interior of the cell. Cilium formation may, therefore, become a useful end point signaling exposure to genotoxins or aneugens. Here we used the aneugen Zidovudine (AZT), an antiretroviral drug that induces DNA replication arrest and centrosomal amplification (CA, >2 centrosomes/cell). Retinal epithelial (RPE) cells grown in labteck-chamber mounted slides were exposed continuously to 0 or 20 μM AZT for 4 days, then the drug was removed and cells were cultured for 48 hr to allow the centrioles to develop cilia. Cells were stained with anti-acetylated tubulin antibody to visualize cilia, anti-pericentrin antibody to visualize centrosomes and DAPI to stain DNA. One hundred cells were scored per treatment revealing 0% and 21.7% of cells (p=0.02) with CA in RPE cells treated with 0 or 20 μM AZT, respectively. Cilliogenesis scoring revealed 48% and 58% of RPE cells with cilia, in cells treated with 0 or 20 μM AZT, respectively. Multiple cilia were observed in 48% of cells in the AZT treated group, about half of the cells producing cilia (p=0.005). Only 8% cells with centrosomal amplification had multiple cilia. Although most cilia were large and robust, short cilia were also present. These preliminary observations suggest that cells with CA are also able to generate cilia. The integrity and functionality of the cilia has yet to be determined.

2739 Influence of Genetic Markers on Exposure Assessment Models

K. Y. Sun[2], J. E. French[2], J. Boyer[2] and L. Nylander-French[2]. [1]*Center for Pharmacogenomics and Individualized Therapy, University of North Carolina at Chapel Hill, Chapel Hill, NC and* [2]*Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC.* Sponsor: L. Nylander-French.

Significant differences in systemic response to xenobiotic exposure result from inter-individual genetic variation, but this variation is not included as a predictor of outcome in current exposure assessment models. We developed an approach to investigate and identify individual differences in genetic variation that influence biomarkers of exposure levels. Urine biomarker 1,6-hexamethylene diamine (HDA) was measured as a quantitative biological phenotype in a well-characterized population of automotive spray painters exposed to 1,6-hexamethylene diisocyanate (HDI). Our innovative statistical modeling approach contains whole-genome markers along with other exposure predictors to determine the contribution of individual genetic variants to the observed urine biomarker levels among the exposed workers. The workers (n=56) were genotyped using genome-wide Affymetrix 6.0 microarrays, which feature ~1.8 million genetic markers. We identified 26 significant genome-wide variants that were associated with the urine HDA levels. Associations were adjusted for multiple comparisons at a false discovery rate (FDR) p<0.05. We also performed a candidate gene analysis using 19 genes encompassing 296 genetic markers associated with HDI exposure and occupational asthma, though none were significant at FDR<0.05. The genetic contribution to variation observed in urine biomarker levels was determined using linear mixed-effects models (LMM) that accounted for personal HDI exposure, individual exposure determinants, and whole-genome polymorphic markers. The biological relevance of significant variants was determined through predictive network analysis. The most significant genetic marker, rs2697962 (p=9.0E-09), was also highly significant in the LMM (p<0.0001). This marker is located in the 3′-UTR region of PRDM2, which encodes a zinc finger protein that is a member of a nuclear histone/protein methyltransferase family.

2740 Antitumoral Monoclonal Antibodies (mAB): Biomarkers for Efficacy and Safety

E. Giannotti, M. Magistrelli, G. Pennella, F. Fiorentini, C. Arrigoni and C. I. Bernardi. *Accelera Srl, Nerviano Milano, Italy.*

Despite the availability of new, more selective and more potent drugs, the successful treatment of hematological malignancies still presents serious challenges. In the past few years, several pharmaceutical companies have been developing mABs targeting specific antigens expressed in hematological malignancies. Aim of this study was to investigate the effect of such mABs on lymphocyte subpopulations and on cytokines in order to identify possible biomarkers for pharmacological and/or toxicological effects. Cynomolgus monkeys were treated intravenously, once or once a week for 4 weeks, with different doses of a bi-specific antibody. Blood samples for mAB plasma levels, lymphocyte subpopulations (by FACS) and cytokines (by Multiplex) were drawn up to 21 days after single administration and at different time points following repeated treatment. Results showed a decrease in B lymphocytes and plasma cells and an increase in activated T lymphocytes, and an increase in some interleukin (IL-2, IL-6, IL-10, IFN-γ and TNF-α), that was completely recovered within 7 hours from treatment for IL-2 and TNF-α and within 24 hours for IL-6, IL-10 and IFN-γ. In conclusion, lymphocyte subpopulations and cytokines can be a useful biomarker for the activity of mABs directed against malignant blood cells.

2741 Anti-Factor Xa: A Surrogate Marker in the Preclinical Assessment of Low Molecular Weight Heparins (LMWHs)

F. Mancari, L. Nobili, D. Lamparelli, C. I. Bernardi and C. Arrigoni. *Accelera Srl, Nerviano Milano, Italy.*

Low molecular weight heparin (LMWHs) is a class of anticoagulant medications, widely used in the prevention and treatment of different severe diseases characterized by venous thromboembolism (ex. deep vein thrombosis and pulmonary embolism) and in the treatment of myocardial infarction. Whilst heparin derived from natural sources (mainly porcine intestine or bovine lung) consists of molecular chains with molecular weights varying from 5000 to over 40,000 Daltons (Da), LMWHs obtained by various methods of fractionation or depolymerisation of polymeric heparin consist of only short chains of polysaccharide having an average molecular weight of less than 8000 Da showing pharmacological effects more predictable than natural or unfractionated heparin. Although approval of new (biosimilar) LMWHs by the FDA is limited on analytical and pharmacological criteria to establish their authenticity, efficacy and safety studies are still required by the European Medicines Agency (EMA) which considers LMWHs as biological products in nature consisting of not fully characterised polysaccharide mixtures. Major issues to be addressed during preclinical studies on LMWHs include the prediction of their effects on the coagulation path, in parallel with robust pharmacokinetics assays. To better characterize a novel LMWH, plasma levels were assessed in parallel with anti-Factor Xa activity on blood samples collected at several time-points from CD-1 male mice given a single intravenous administration of LMWH (6 mg/kg). The anti-Factor Xa activity was measured using the chromogenic assay Berichrom® Heparin Kit adapted on Berichrom Coagulation System (BCS) analyzer, whilst plasma concentrations were determined using the Heparin Red® (RedProbes, Germany) fluorescence quenching homogeneous assay method, followed by standard pharmacokinetics calculations. The results obtained demonstrate a good correlation (0.872) between LMWH concentrations in plasma measured by using the Heparin Red assay method and the anti-Factor Xa activity.

2742 N^6-Formyllysine as a Biomarker of Formaldehyde Exposure in Non-Human Primates

B. Edrissi[3], K. Taghizadeh[3], R. Yu[4], B. Moeller[1], D. Kracko[1], M. Doyle-Eisele[1], J. A. Swenberg[4] and P. C. Dedon[3]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM;* [2]*Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, MA;* [3]*Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA and* [4]*Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC.*

N^6-Formyllysine, a chemical homolog of the epigenetically important N^6-acetyllysine, is a global pathological protein modification with formaldehyde as its major source. Our previous *in vivo* studies in rats exposed by inhalation to [13C2H2]-formaldehyde showed dose-dependent formation and accumulation of this adduct in nasal mucosa but not in tissues distant to the site of entry. However, structural differences in nasal passages of rodents and primates and their breathing patterns make primates a more appropriate model to study formaldehyde exposure. To this end, we quantified endogenous and exogenous N^6-formyllysine adducts in several tissues of cynomolgus macaques that were whole body exposed to 2 ppm [13C2H2]-formaldehyde vapor (6 hrs/day for 2 days) using liquid chromatography-coupled tandem mass spectrometry. There were 3–10 endogenous adducts per 104 lysines depending on the tissue type. However, exogenous adducts were only detected in nasal tissues (maxilloturbinates, septum, nasopharynx, and dorsal region) and trachea, but not in lung, bone marrow, or white blood cells. Furthermore, exogenous adducts at best contributed to 3% of the endogenous ones. These results are consistent with previous studies in rodent models of formaldehyde exposure and point to a risk for formaldehyde exposure limited to the upper airway. The widespread nature of the N^6-formyllysine adducts raises questions about their effects on epigenetic regulation of gene expression and about the mechanisms of formaldehyde toxicity and carcinogenicity.

2743 Dual Regulation of Calcineurin Activity Following Lymphocyte Activation: Involvement of an Endogenous Mechanism and Contribution to Cyclosporine-Induced Toxicity

C. Lena[2], L. Herry[2], C. Tomkiewicz-Raulet[2], R. Barouki[1,2] and S. Sanquer[1,2]. [1]*Hôpital Necker-Enfants malades, Paris, France and* [2]*Paris Descartes University, Paris, France.*

Introduction: The standard for rejection prevention in transplantation includes a calcineurin (CN) inhibitor, such as cyclosporine. Although it was thought that low CN activity reflected excessive immunosuppression that would protect against rejection but facilitate the development of adverse events, we have recently found that both rejection and CsA-related adverse events were paradoxically accompanied by a decreased CN activity. In addition, we have found that lymphocyte activation *in vitro* leads to a fast but transient stimulation of CN activity that is gradually decreased at late time points. The aim of this study was to provide a molecular explanation for this dual regulation of CN activity. Methods: We measured CN activity in human lymphocytes up to 48 h following stimulation by PMA/ionomycin by using tandem mass spectrometry. The expression of CN catalytic unit (CN-A) and regulator of CN1 (RCAN1) were assessed by RT-QPCR and western blot. Co-immunoprecipitation (co-IP) experiments were performed to study the interactions between CN-A and RCAN1. Results: Significantly lower levels of CN-A transcripts were recovered after 7 h stimulation with PMA/ionomycin and remained low up to 48 h after stimulation. This effect was confirmed at the protein level with significantly reduced CN-A expression after 24 and 48 h stimulation. In contrast, significantly higher levels of RCAN1 transcripts were found after 7 h stimulation and were maintained up to 48 h after stimulation. This effect was also confirmed at the protein level with significantly increased RCAN1 expression after 24 and 48 h stimulation. Increased interactions between CN-A and RCAN1 were found up to 7 h after lymphocyte stimulation. Conclusion: Our results confirm the dual regulation of CN activity following *in vitro* lymphocyte activation since the expression of CN-A was dramatically reduced at late time points after lymphocyte activation. Furthermore, the increased expression of RCAN1 and its increased interaction with CN-A could explain the decreased CN activity after lymphocyte activation. We anticipate that the activation of such endogenous mechanism following lymphocyte activation could contribute to the toxicity induced by cyclosporine in patients, especially as the drug dosage is increased when rejection is suspected.

2744 Imposex in *Stramonita haemastoma* from Coastal Sites of Cartagena, Colombia

L. Sierra-Marquez, J. Sierra-Marquez and J. Olivero-Verbel. *School of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia.*

Imposex is the development of male sexual characteristics in females, a problem caused by toxic effects of some substances that act as endocrine disruptors. Antifouling paints contain these chemicals. The Caribbean Coast of Colombia lacks studies to indicate the extent of imposex in organisms from its coastal waters. The aim of this study was to determine the prevalence of imposex in the gastropod *Stramonita haemastoma* in Cartagena, Colombia. Specimens were collected from locations of high and low influence of port activity in 2013. Several morphometric measurements were performed to evaluate the frequency and characteristics of the occurrence of imposex. Morphometric variables showed statistically significant differences between the specimens from the two studied sites. The female specimens of *Stramonita haemastoma* presented an imposex frequency of 93.1% in Isla de los Pájaros, compared to 31.8% in La Bocana, a reference location. The relative penis size index or RPLI (10.145 and 3.231) and the vas deferens sequence index or VDSI (2.83 and 1.16), suggested possible contamination by organotin compounds in both places. The rates of incidence of imposex found in the city of Cartagena are a clear biomarker of exposure and biological effects caused by TBT in coastal areas associated with influence of port activities. Vice-Presidency for Research of the University of Cartagena (Grants 2012–2014) and National Program for Doctoral Studies in Colombia (Colciencias, 647-2014).

2745 A Highly Sensitive and Stable Biomarker of Oxidative Stress

T. Shimomoto, L. Collins, X. Yi and J. Nakamura. *Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC; Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Malondialdehyde (MDA) is one of the most widely used biomarkers of oxidative stress. MDA has often been quantitated using the TBARS assay; however, this assay lacks specificity and sensitivity. Interestingly, reactions involving two molecules of MDA, one molecule of acetaldehyde (AA), and the lysine on proteins lead to the formation of very stable MDA-AA (MAA)-protein adducts. MAA-protein adducts are widely reported to be produced upon oxidative stress. Furthermore, these protein adducts have high immunogenicity, proinflammatory and profibrogenic effects. However, all previous reports utilized crude MAA protein products for various analyses including antibody titer analysis. The crude MAA products include a mixture of many different protein adducts caused by MDA and AA, resulting in lack of specificity and sensitivity. Here, we have succeeded in purifying MAA adducts using HPLC. We found that MAA-protein adducts form under physiological pH and temperatures. BSA conjugated with purified MAA (pMAA-BSA) showed immunogenicity in mice without adjuvant, and antibodies that have been reported to be specific to MAA reacted with our pMAA-BSA. To compare the sensitivity of pMAA-BSA versus crude MAA-BSA, we used non-competitive ELISA assays to detect serum antibody titers of these adducts in wildtype and atherosclerosis-prone ApoE-/- mice. First, the anti-MAA antibody levels in ApoE-/- mice were much higher than those in wildtype. Second, the difference between the antibody titers in the two groups was much greater in the pMAA-BSA ELISA assay than that in the crude MAA-BSA assay. These results indicate that the pMAA-BSA ELISA assay has much higher sensitivity than the crude MAA-BSA assay. Antibody titer measurement requires less than 1 µl of blood samples, a volume that is feasible for assaying clinical and epidemiological samples. Together, our findings reveal a highly sensitive method to detect antibody titers of pMAA-protein adducts, which can serve as a valuable and stable biomarker of oxidative stress.

2746 The Role of Micrornas in Regulation of Drug Metabolizing Enzymes and Transporters

B. Ning[2], D. Yu[2], W. Tolleson[2], B. Knox[2], Y. Guo[1], Y. Jin[1], S. Kadlubar[3], I. Pogribny[2] and J. Fuscoe[2]. [1]*Beijing Children's Hospital, Capital Medical University, Beijing, China;* [2]*NCTR/US FDA, Jefferson, AR and* [3]*University of Arkansas for Medical Sciences, Little Rock, AR.*
Sponsor: B. Ning.

Observed variations in drug responses among patients may result from differences in heritable genetic traits or from alterations in the epigenetic regulation of drug metabolizing enzymes and transporters (DMETs). MicroRNAs (miRNAs) are a family of small (usually 20-24 nucleotides long) non-coding RNAs comprising up to 2% of mammalian genomes. MiRNAs can regulate gene expression post-transcriptionally, providing an epigenetic mechanism for fine-tuning the expression of targeted DMET genes by regulating the efficiency of protein translation and by decreasing mRNA stability via enhanced degradation. In recent studies, we systematically screened a group of important DMET genes for their potential response elements to miRNAs. These DMET genes include CYP2C9, CYP2C19, CYP2B6, SULT1A1, ALDH5A, ABCC6 and SLC22A7. RNA electrophoresis mobility shift assays displayed direct interactions between microRNAs and their cognate targets within the mRNA transcripts for the genes. The expression of luciferase reporter genes containing the transcripts of these DMETs and the expression of endogenous DMETs were each suppressed by transfection with corresponding miRNAs. Importantly, chemically-induced up-regulation of related miRNAs correlated inversely with the expression of these DMET genes. Association analyses revealed that the expression of miRNAs is significantly inversely correlated with the expression of these DMETs in human tissues. Our studies demonstrated that, as important pharmacogenomic components, miRNAs manifest characteristics of inter-individual variability in two aspects: (a) genetic polymorphisms altering binding sites for miRNAs in the mRNA transcripts they regulate and (b) variability in miRNA expression among the population.

2747 MicroRNA Hsa-miR-25-3p Suppresses CYP2B6 in Human Liver Cells

D. Yu[2], Y. Jin[1], W. Tolleson[2], B. Knox[2], Y. Guo[1], S. Chen[2], L. Guo[2] and B. Ning[2]. [1]*Beijing Children's Hospital, Beijing, China and* [2]*National Center for Toxicological Research, Jefferson, AR.* Sponsor: B. Ning.

Cytochrome P450 2B6 (CYP2B6), mainly expressed in the liver and brain, is one of the important drug metabolizing enzymes (DMEs) involved in metabolism of many drugs, and homeostasis of cholesterol, steroids and lipids. Studies have demonstrated that genetic variants are involved in differential expression of CYP2B6 among human populations. However, this explains only a portion of the inter-individual variability in CYP2B6 expression. In this study, we performed in sillico, *in vitro* and *in vivo* analyses systematically to identify the regulatory mechanisms of microRNAs (miRNAs) in CYP2B6 expression. *In silico* analyses showed that hsa-mir-25-3p and hsa-mir-504-5p are potential modulators regulating CYP2B6 expression. RNA electrophoresis mobility shift assays (EMSAs) demonstrated a direct interaction between hsa-mir-25-3p and 3'-UTR of the CYP2B6 transcript. In addition, we found that the Ago4 protein, but no other AGO family members, could facilitate the interaction between hsa-mir-25-3p and its cognate target, to form a multi-protein RNA-induced silencing complex (RISC). Further, we observed that the up-regulation of CYP2B6 expression induced by rifampicin or cyclophosphamide could be eliminated by transfection of hsa-mir-25-3p mimics. Finally, an inverse correlation between CYP2B6 and hsa-mir-25-3p expression was observed in a cohort of human liver tissue samples. In conclusion, our results indicated that hsa-miR-25-3p can suppress CYP2B6 expression in human liver cells, in an Ago4-dependent manner.

2748 Polymorphisms and Expressions of GSTM1 and GSTT1 in Human Non Small Cell Lung Cancer

M. Iscan[6], M. Kilic[6], A. O. Ada[6], S. Oguztuzun[4], F. Demirag[3], S. Celik[2] and P. Bicakcioglu[1]. [1]*Ataturk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey;* [2]*Yildiz Technical University, Istanbul, Turkey;* [3]*Department of Pathology, Ankara Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey;* [4]*Faculty of Arts and Sciences, Department of Biology, Kirikkale University, Ankara, Turkey;* [5]*Faculty of Pharmacy, Toxicology, Ankara University, Ankara, Turkey and* [6]*Vocational School of Health Sciences Department of Pharmacy Services, Ankara University, Ankara, Turkey.*

The deletion polymorphisms in genes encoding glutathione S-transferases (GSTs) M1 and T1 result in the absence of the corresponding enzyme, decreasing detoxification of tobacco carcinogens. In this study, we aimed to demonstrate whether a) polymorphisms and protein expressions of GSTM1 and GSTT1 isoenzymes match or not in lung tissues of patients with non-small cell lung carcinoma (NSCLC) b) the absence of one GST influence the expression level of the other GST, and c) there exists GSTM1 and GSTT1 protein expression differences between tumor tissues and normal tissues of patients with GSTM1+ and GSTT1+ gene. DNA was isolated from paraffin-embedded archive tissues from 33 NSCLC patients for polymorphisms of these isoenzymes were studied using multiplex-PCR. Protein expressions of GSTM1 and GSTT1 were examined by immunohistochemistry. GSTM1 and GSTT1 protein expressions were not detected in the patients with GSTM1 and GSTT1 gene deletions whereas protein expression was detected in tumor and normal tissues of all patients with GSTM1+ and GSTT1+ gene. The protein expression level of GSTT1 was 2.0-fold higher particularly in tumors of patients lacking GSTM1 gene than those having GSTM1 gene ($p=0.018$). Protein expression of GSTM1 was statistically higher in tumor tissues than in normal tissues in patients with GSTM1+ gene ($p=0.001$) whereas no significant difference was noted for GSTT1 expression ($p>0.05$). The results show that a) there is an association between gene deletions and protein expression levels of GSTM1 and GSTT1 in lungs of patients with NSCLC, b) in the absence of GSTM1 gene, GSTT1 isoenzyme is likely to take also the responsibility of GSTM1 isoenzyme in detoxification of toxic electrophiles, and c) overexpression of GSTM1 protein in tumor compared to normal lung tissue could render it to be a useful tumor marker for NSCLC.

2749 Maternal Genotype for Arsenic (+3 Oxidation State)-Methyltransferase Influences Inorganic Arsenic Metabolism and Newborn Birth Outcomes

Z. Drobna[2], E. Martin[2], K. S. Kim[2], M. Ruio-Andrade[1], G. G. García-Vargas[1], M. Stýblo[2] and R. C. Fry[2]. [1]*Facultad de Medicina, Universidad Juarez del Estado de Durango, Gómez Palacio, Durango, Mexico and* [2]*Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC.* Sponsor: R. Fry.

High levels of inorganic arsenic (iAs) in drinking water and inefficient methylation of iAs are known to be associated with adverse health outcomes. However, very little is known about the role of single nucleotide polymorphisms (SNPs) in genes involved in iAs methylation of pregnant mothers and newborn birth outcomes. The objective of this study was to evaluate the role of the arsenic (+3 oxidation state) methyltransferase (AS3MT) gene variants on the metabolism of iAs by pregnant women of the Biomarkers of Exposure to ARsenic (BEAR) cohort in Gomez Palacio, Mexico, and its effects on newborn birth outcomes. Seven SNPs in AS3MT were analyzed with respect to the concentration of inorganic arsenic in drinking water and urinary maternal concentrations of iAs and methylated arsenic metabolites in the urine of the women and the newborn birth outcomes using multiple variable linear regression. Overall we found 5 out of 7 SNPs of AS3MT to be associated with urinary maternal concentrations of arsenic metabolites. SNP rs3740393 showed an association with both changes in maternal metabolism and birth outcomes. In addition, when stratified by baby sex, mothers who were pregnant with males showed a more significant correlation between maternal SNPs of AS3MT and both maternal arsenic metabolite concentrations and birth outcomes. AS3MT polymorphisms appear to affect the metabolism of arsenic in pregnant mothers and to smaller degree birth outcomes.

2750 FDALabel Database: A Rich Resource for Study of Pharmacogenomics Biomarkers in Advancing Drug Safety and Precision Medicine

H. Fang, J. Xu, Z. Liu, Y. Liu, S. Harris, S. Thakkar, G. Zhou, G. Zhang, P. Howard and W. Tong. *National Center for Toxicological Research, US FDA, Jefferson, AR.*

Pharmacogenomics (PGx) is a study of correlating benefit or adverse effects of drug treatment with individual genetic differences. Understanding the association between PGx markers and phenotypes improves knowledge of underlying mechanisms of diseases and treatment responses for enhanced drug safety and precision medicine. Research on this topic has been a challenge, because of lack of easy access to PGx data. We report here the FDALabel database (www.fda.gov/scienceresearch/bioinformaticstools/ucm289739.htm), which allows users to perform customizable, full-text searches in over 70,000 drug labeling documents. FDALabel provides PGx information contained in the FDA-approved drug labeling (package insert). The drug insert information provides the official consensus and combined information about product indications, target populations, and adverse drug reactions (ADRs) from regulators, drug manufacturers, and scientific experts. In this study, biomarker information was used as query on the relevant PGx sections in FDALabel. As a result, we identified more than 170 drugs out of ~1600 small molecule drugs with genetic biomarker information. Furthermore, 36 biomarkers were identified and divided into three categories (1) drug metabolism variability (e.g., CYP enzymes); (2) increased risk of adverse events (e.g., G6PD, TPMT, HLA-B); (3) drug's mechanism of action (e.g., CD30). These biomarkers are likely to impact the effect of drug response to the specified patient sub-population. Network analysis and visualization were used to illustrate the relationship amongst drugs, biomarkers, and associated adverse effects. In summary, the use case of PGx biomarkers has demonstrated the potential of using FDALabel for the study of ADRs (e.g., to identify new trend and frequency of genetic variability associated with increased risks to public health) in pursuit of improved pharmacovigilance and precision medicine.

2751 Quantification and Genetic Components of Synergistic Interactions among Anticancer Drugs in Lymphoblastoid Cell Lines

K. R. Roell[3], T. M. Havener[2], J. R. Jack[3], H. L. McLeod[1], D. M. Reif[3] and A. A. Motsinger-Reif[3]. [1]*Moffitt Cancer Center, Tampa, FL;* [2]*University of North Carolina at Chapel Hill, Chapel Hill, NC and* [3]*Bioinformatics Research Center, North Carolina State University, Raleigh, NC.*

The current paradigm in chemotherapy is often to prescribe multiple drugs at once. However, the mechanism and inter-individual responses to these drugs are most often studied and developed independently, despite clinical evidence that drugs do not act independently when co-administered. Such synergistic (or antagonistic) effects occur between chemicals when the observed effect of the combination is more (or less) than what would be predicted from the effects of each agent working alone (both in the drug and chemical contexts). Modeling synergistic interactions presents interesting questions from both quantitative modeling and underlying biological perspectives. There are a number of statistical methods for modeling synergy from a qualitative perspective, but rigorous quantification and detection of synergy, specifically within drug/chemical mixtures, is limited. Additionally, more investigation is needed into underlying mechanisms of synergistic effects, particularly in regards to potential genetic etiology. We have taken a novel approach to study synergy between chemotherapeutic drugs by applying known methods to show that synergy is heritable in a proven model of cytotoxic response using lymphoblastoid cell lines (LCLs). We used the Chou-Talalay combination index approach to quantify synergy and results suggest interactions among common chemotherapy drugs. We then used these results to test for a genetic component of variation in synergistic response. We identified significant genetic components (> 50% heritability) among several drug combinations. As this indicates a genetic basis for synergistic response, we are performing genome-wide association studies to identify candidate genes that explain a significant amount of synergistic variation.

2752 Deletion of Catalase in C57BL/6 Mice Results in Obese Phenotype and Metabolic and Morphologic Changes

C. Heit[3], S. Marshall[1], G. Charkoftaki[1], D. Orlicky[3], K. Fritz[3] and V. Vasiliou[1]. [1]*Yale University, New Haven, CT;* [2]*Environmental Health, Yale University, New Haven, CT and* [3]*Pathology, University of Colorado, Aurora, CO;* [4]*Pharmaceutical Science, University of Colorado, Aurora, CO.*

Hydrogen peroxide is a toxic agent for many living organisms, resulting in oxidative stress and cellular damage. Catalase is an enzyme involved in the metabolism of hydrogen peroxide, acting as a defender against oxidative stress and also metabolizes ethanol to acetaldehyde, using hydrogen peroxide as a cofactor. A subset of the human population, who have inherited acatalasemia, the deficiency in catalase activity primarily in red blood cells, have higher risk for numerous pathologies, including diabetes. Mice with global lack of catalase (catalase knockout mice or CKO) are susceptible to streptozotocin induced diabetes and diabetic renal injury. Our research has shown that catalase knockout mice have increased oxidative stress and a distinctly obese phenotype. Both male and female mice exhibit an increase in body weight from fat accumulation, a phenotype that gets more defined with age. Females displayed a more pronounced body fat accumulation, whereas male mice tended to accumulate more fat in the liver rather than viscerally. Both sexes have increased serum and liver triglycerides as well as ALTs. Both sexes exhibit hypertrophic adipocytes although females show crown structures whereas male do not. Pancreatic histology also indicates larger islets, which is corroborated by increased fasting insulin levels. These preliminary findings indicate that catalase plays a more significant role in metabolic disorders and fatty liver than originally thought.

2753 Genetic Background Plays a Role in Risk of Zileuton-Induced Liver Injury in Diversity Outbred Mice

L. E. Lyn-Cook[2], D. Gatti[1], G. Churchill[1] and A. Harrill[2]. [1]*The Jackson Laboratory, Bar Harbor, ME and* [2]*University of Ark for Medical Sciences, Little Rock, AR.*

Zileuton is a first line medication for maintenance treatment of asthma; however, its use is associated with a rare ("idiosyncratic") incidence of severe drug-induced liver injury (DILI). There is a lack of animal or *in vitro* models for zileuton DILI, thus identification of a model system that can be used to facilitate mechanistic understanding and identification of biomarkers for personalized medicine is desirable. A critical issue is that conventional models lack genetic diversity, which in several instances has been shown to play a role in adverse drug reactions. The Diversity Outbred (DO) mice comprise a genetically diverse population with variability that surpasses that of the human population, but in which the minor allele frequency is greater in the DO (12.5% on average). Pilot studies previously confirmed the suitability of DO mice for investigation of zileuton DILI as elevations in ALT and miRNA-122 were observed in a subset of genetically sensitive mice. Therefore, the study was expanded to include a sufficient number of mice to power a pharmacogenetic analysis. In this study, female DO mice (N=400/group) were administered zileuton orally (600 mg/kg/day for 7 days) or vehicle (N=50). Blood samples were taken before dosing ("baseline") and at necropsy. While variability was observed in clinical chemistry markers within each cohort, reference ranges were established from the vehicle control group. As compared to vehicle, at necropsy there was significant elevation in ALT, AST, and bilirubin in the zileuton treated group ($P<0.05$). There was no significant difference with ALP and BUN ($P>0.05$). In the vehicle control group, analyte ranges and medians were: ALT (16.0-71.0; 27.50), AST (52.0-272.0; 76.0), and bilirubin (0.1-0.5; 0.2). In contrast, in the zileuton treated mice, the analyte ranges and median were ALT (14.0-481.0; 39.0), AST (45.0-765.0; 93.0), and bilirubin (0.1-4.6; 0.2). ALT elevations in zileuton-treated mice were accompanied by histopathological evidence of hepatocellular injury. Thus, we conclude that the DO mice are an appropriate model for the study of zileuton DILI and provide the opportunity to investigate pharmacogenetic risk factors for sensitivity.

2754 RNA-Seq Reveals Age- and Species-Differences of CAR in Regulating the Drug-Processing Genes in Liver

S. L. Cheng. *University of Washington, Seattle, WA.* Sponsor: J. Y. Cui.

Newborns are considered more vulnerable to xenobiotic insults, because relatively less is known regarding the pharmacological regulation of various drug-processing genes during liver development. The constitutive androstane receptor (CAR/Nr1i3) is an important xenobiotic-sensing nuclear receptor that is highly expressed in liver. It has been shown that there are species-differences between mouse (m) and human (h) CAR in modulating the hepatic cell proliferation, but there lacks a systematic comparison between mCAR and hCAR on the pharmacological regulation of drug-processing genes. Therefore, the goal of this study was to use RNA-Seq to unveil the potential age- and species-differences of CAR activation on the expression of the hepatic transcriptome. At either 5- or 60-days of age, wild-type, CAR-null, and humanized-CAR transgenic (hCAR-TG) mice were administered a species-appropriate CAR-ligand (TCPOBOP for mCAR, and CITCO for hCAR), or vehicle, once daily for 4-days (i.p.). RNA-Seq was performed in wild-type and hCAR-TG mice, whereas and RT-qPCR validation was conducted in all groups of mice. Unique age- and species-specific regulatory patterns of the hepatic drug-processing genes were identified: 1) common targets of both mCAR and hCAR at both ages (Cyp2b10, Cyp3a11, Gstm1, Sult1e1, Ugt1a9, and Mrp2), and in general, neonatal livers had greater fold-increase in the expression of these genes in a CAR-dependent manner; 2) up-regulated by both mCAR and hCAR at one age only (Cyp1a2 and Sult5a1 up-regulated only in newborns, and Sult3a1 up-regulated only in adults); 3) up-regulated by mCAR but not hCAR (Nqo1, Aldh1a1, Gstm2, Gstt1, Papss2, Ugt2b34, Ugt2b36, and Mrp4). The differential expression of selected DPGs was confirmed by western blot and enzyme activity assays, and the hCAR-specific effect was confirmed in HepaRG cells. In conclusion, neonatal livers are more sensitive to CAR-activators, and there are species-differences of CAR in modulating the drug-processing gene expression in liver.

2755 Toxicological Assessment of Multi-walled Carbon Nanotubes *In Vitro*: Potential Mitochondria Effects on Male Reproductive Cells

A. Gu. *School of Public Health, Nanjing Medical University, Nanjing, China.*

Multiwalled carbon nanotubes (MWCNTs) have been widely used in many fields including material engineering and biological medicine over the past decade. One previous study found that MWCNTs could penetrate blood-testis barrier in mice and damaged seminiferous tubules. However the potential reproductive effects of 'nontoxic' doses MWCNTs exposure on an effectively *in vitro* assay remained elusive. Herein, we used the mice Sertoli cell line (TM-4) and spermatocyte cell line (GC2-spd) to assess the male reproductive effects of MWCNTs. In this study, size distribution, zeta potential, and intensity of MWCNTs are characterized. MWCNTs is suspended with different concentrations and toxicological investigations are carried on both cell lines. A max-

imal nontoxic dose of 0.5 µg/ml was found in both cell lines, without altering the parameter of nuclear size, cell permeability, mitochondrial membrane potential, and cytochrome C as well. At this dose, cell cycles, apoptosis and the ROS levels were in nomal status. Interestingly, even at the nontoxic dose, MWCNTs were found to accumulate in mitochondria, which caused mitochondrial DNA damage in both cell lines. Furthermore, the expression of mitochondria-related genes, the oxygen consumption rate, and cellular ATP content declined compared to controls, even at the 'nontoxic' MWCNTs dose group. Our results suggested that mitochondrion was a specific organelle that accumulates MWCNTs, and a sensitive cellular target that responsed to MWCNTs exposure as well. The findings increase our understanding of the potential mitochondrial effects of MWCNTs on male reproduction even at low doses, and could have important implications for nanotoxicity testing *in vitro*.

2756 Unique Nanoparticle Optical Properties Confound Fluorescent Based Assays Widely Employed in Their *In Vitro* Toxicity Screening and Ranking

K. Dreher[1] and W. Polk[2]. [1]*US EPA, Research Triangle Park, NC and* [2]*Curriculum in Toxicology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Nanoparticles (NPs) are novel materials having at least one dimension less than 100 nm and display unique physicochemical properties due to their nanoscale size. An emphasis has been placed on developing high throughput screening (HTS) assays to characterize and rank the toxicities of chemicals in a manner consistent with the vision of the Toxicology in the 21st century Vision. Research reported here demonstrates that many of the available HTS fluorescent based assays can be confounded by metal oxide NPs and that their use without careful controls or adequate adjustments can result in both false positive and false negative errors. Effects are demonstrated for assays measuring cytotoxicity, oxidative stress, and nuclear content using commercially available metal oxide NPs ranging in size from 7 to 250 nm and varying in composition as well as crystalline structure. NP optical confounding effects are demonstrated in a variety of primary human endothelial cell types and are shown to be more severe within cells than would be anticipated from analytical analysis of NP dispersions alone. The effects are demonstrated to be NP dependent and not predictable by elemental composition, purity, or primary particle size. In addition, NP optical properties were found to interfere with the ability to determine the extent to which oxidative stress contributed to titanium oxide NP toxicity within endothelial cells. It is concluded that data generated by HTS assays have the potential to be impacted by NP optical interference requiring adjustments to be made for each NP within an *in vitro* testing system prior to the initiation of toxicity screening. (This abstract does not represent US EPA policy. Research partially funded by the US EPA - UNC Cooperative Training Agreement CR-83515201)

2757 Toxicity Study of Mycosynthesised Silver Nanoparticles on *Pseudomonas putida*

I. R. Gupta[2], A. J. Anderson[1] and M. K. Rai[3]. [1]*Department of Biology, Utah State University, Logan, UT;* [2]*Department of Biotechnology, Institute of Science, Aurangabad, India and* [3]*Department of Biotechnology, S. G. B. Amravati University, Amravati, India.* Sponsor: U. Apte.

Silver nanoparticles has many applications due to their advantageous properties. But with rise in their use there is risk of development of hazardous effects to the human and environment. With reference to many studies performed *in vitro* and *in vivo* there are various toxicological effects of silver nanoparticles on eukaryotes and prokaryotes. Therefore, there is an urgent need of studying the toxicological aspects of silver nanoparticles to microbes which are helpful for maintaining the environment. In the present study we made an attempt to assess the toxicity of biologically synthesized silver nanoparticles on *Pseudomonas putida*, a beneficial soil microbe. For this study we synthesized silver nanoparticles by using various fungi, followed by their characterization through UV-vis spectrophotometry, FTIR, X-ray diffraction, nanosight LM20--a particle size distribution analyzer and TEM. By using soil bacterial biosensor generated in *P. putida* we have investigated the toxicity of silver nanoparticles. The results suggest the inhibition of silver nanoparticle exposed *P. putida* thereby showing the hazardous effect on soil environment.

2758 Differential Genotoxicity Mechanisms of Silver Nanoparticles and Silver Ions

T. Chen[2], Y. Li[2], T. Qin[2], T. Ingle[2], J. Yan[2], W. He[1] and J.-J. Yin[1]. [1]*US FDA/FSAN, College Park, MD and* [2]*US FDA/NCTR, Jefferson, AR.*

In spite of many reports on the toxicity of silver nanoparticles (AgNPs), the mechanisms underlying the toxicity are far from clear. A key question is whether the observed toxicity comes from the silver ions (Ag+) released from the AgNPs or from the nanoparticles themselves. In this study, genotoxicity and its mechanisms of Ag+ and AgNPs were explored. Human TK6 cells were treated with 5 nm AgNPs or silver nitrate (AgNO3) to evaluate their genotoxicity and induction of oxidative stress. AgNPs and AgNO3 induced cytotoxicity and genotoxicity at a similar range of concentration (1.00 -1.75 µg/ml) when evaluated using the micronucleus assay; and both of them induced oxidative stress by measuring the gene expression and reactive oxygen species in the treated cells. Addition of N-acetylcysteine (NAC, an Ag+ chelator) to the treatments significantly decreased genotoxicity of Ag+, but not AgNPs, while addition of Trolox (a free radical scavenger) to the treatment efficiently decreased the genotoxicity of both the agents. In addition, the Ag+ released from the highest concentration of AgNPs used for the treatment was measured. Only 0.5% ions were released from the AgNPs in the culture medium and the released silver ions were neither cytotoxic nor genotoxic at this concentration. Further analysis using electron spin resonance demonstrated that AgNPs produced hydroxyl radicals directly, while AgNO3 did not. These results suggested that although both AgNPs and Ag+ can cause genotoxicity via producing oxidative stress, the mechanisms are different and the nanoparticles, but not the released ions, mainly contribute to the genotoxicity of AgNPs.

2759 RBCs as Surrogates for Studying Lysosomal-Nanoparticle Interactions and Nanoparticle Toxicity

L. Fisch. *Chemistry and Biochemistry, Montana State University, Bozeman, MT.* Sponsor: A. Holian.

Engineered nanomaterials (ENMs) are a new class of materials that are being developed for a wide variety of uses in fields such as medicine, engineering, and personal products increasing the potential for human exposures and possible adverse effects. However, the rapid pace of development of new ENMs has precluded extensive testing of all materials. Recent work has demonstrated in various cell models that bioactive ENMs are taken up by endocytosis and processed in the lysosome. Some nanoparticles are believed to cause toxicity by lysosomal membrane degradation resulting in inflammatory pathway activation, reactive oxygen species production, mitochondrial damage, and cell death. Since there are many different types of ENMs a high throughput assay was developed to test the toxicity of ENMs using red blood cell (RBC) hemolysis as an indicator of toxicity. Membrane degradation of a red blood cell may indicate that the nanoparticle will also cause lysosomal membrane degradation. Furthermore RBC hemolysis will bring to attention the necessity for further toxicity testing of the specific ENM. Purchased human RBC were incubated with particles and the amount of hemoglobin released by the RBCs was quantified. Initial studies were conducted in 1.5 ml tubes then the assay was developed for high throughput using 96 well plates. Pigment grade TiO2, NiO, and TiO2 nanospheres were tested for toxicity with this assay using crystalline silica as a positive control. Pigment grade TiO2, and NiO nanoparticles induced RBC hemolysis while TiO2 nanospheres did not. Additional experiments showed that human serum protected RBCs exposed to silica and NiO from hemolytic damage and that an acidic pH to simulated the lysosomal environment did not reverse these protective effects. This study was supported by NIH grants R25 ES022866-01 and P30 GM103338.

2760 The Effect of Protein Corona Formation on the Intracellular Uptake Profiles of Gold Nanoparticles in Human Primary Vascular Endothelial Cells

P. Chandran and N. A. Monteiro-Riviere. *Nanotechnology Innovation Center of Kansas State, Kansas State University, Manhattan, KS.*

The formation of a protein corona covering nanoparticles (NP) upon entry into the systemic circulation confers a new biological identity thereby, altering their native synthetic identity resulting in various NP-cell interactions, uptake, and biodistribution profiles, subsequently determining their fate *in vivo*. A systematic investigation was conducted on the role of size and surface chemistry on intracellular uptake profiles of 40 nm and 80 nm sized gold NP (AuNP) with branched polyethylene

imine (bPEI), lipoic acid (LA) and polyethylene glycol (PEG) coatings. Hard corona protein profiles of AuNP with various surface functionalization were analysed using SDS-PAGE, after incubating AuNP with pooled human whole blood plasma (n=5) for 1 h. Time dependent intracellular uptake patterns for 15 min, 30 min, 1, 3, 6, 12 and 24 h were studied quantitatively employing inductively coupled plasma-mass spectrometry (ICP-MS), and qualitatively using hyperspectral imaging and transmission electron microscopy (TEM) in human umbilical vein endothelial cells (HUVEC). The protein patterns recovered from the AuNP differred from the plasma control, revealing surface functionalization dependent signature adsorbomes. AuNP exhibited size and surface chemistry dependent uptake patterns, wherein 40 nm bare and protein bound AuNP showed greater uptake compared to 80 nm AuNP, whereas bPEI coated AuNP showed the highest uptake, followed by LA and PEG coatings. Interestingly, protein corona bound AuNP exhibited a reduced cell uptake compared to their bare counterparts, wherein protein bound bPEI coated AuNP induced aggregation, critically affecting their internalization. Even though, protein bound LA and PEG coated AuNP did not induce any aggregation, they showed less cellular uptake. Establishment of definite links between the NP physicochemical characteristics, signature protein corona profiles, and intracellular uptake will benefit suitable designing of nanomaterials for drug delivery applications.

2761 Investigation on the Toxicity of Bismuth Oxide Nanoparticles in HepG2 Cell Line

E. Öztas, M. Abudayyak, M. Arıcı and G. Özhan. *Faculty of Pharmacy, Istanbul University, Istanbul, Turkey.*

Nanoparticles have been increasingly drawn attention in various fields ranging from medicine to industry due to their physicochemical properties and functions. Bismuth based compounds have been commonly used in the industrial, biotechnical, cosmetic and medical applications. Although the toxicity of bismuth compounds were studied for years; there is a serious lack of information concerning the toxicity and effects of Bi based compounds in the nano scale on human health and the environment. Therefore, in the present study, it was aimed to investigate toxic effects of bismuth oxide nanoparticles (average size 149.1 nm) *in vitro* in HepG2 hepatocellular carcinoma cell line. BiO nanoparticles uptake from the cells was studied using the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and nanoparticles uptaken by the cells showed cyto- and genotoxic effects. Cytotoxic activity was determined by MTT and neutral uptake assays based on different cellular mechanisms depending on damaged region, and genotoxicity was determined by comet assay. IC50 values of BiO nanoparticles were 35.11 µg/mL by MTT assay and 92.09 µg/mL by NRU assay. The cell death via apoptosis (early or late) or necrosis was assessed by Annexin V-FITC with PI apoptosis detection assay and it was observed that the main cell death pathway is apoptosis in HepG2 cells. The glutathione (GSH), malondialdenyde (MDA) and 8-hydroxy deoxyguanine (8-OHdG) levels were significantly changed. Levels of protein carbonyl (PC) change were not significant. In conclusion, it should be considered the toxicity of BiO nanoparticles might be different from that of macro-sized Bi compounds due to their large surface area and physicochemical properties. Bi nanoparticles should be carefully and thoroughly used even if the results are needed to support with *in vivo* studies and fully understand the mechanism of their toxicity.

2762 Silver Nanoparticles Induce Pyroptosis by NLRP3-Inflammasome Dependent Caspase-1 Activation in Macrophages

A. Mishra, G. Kumar and P. L. Goering. *CDRH, US FDA, Silver Spring, MD.*

Silver nanoparticle (AgNP)-macrophage interactions and subsequent secretion of pro-inflammatory cytokines are well-documented; however, mechanisms of AgNP-induced macrophage activation and toxicity are not fully elucidated. In the present study, we investigated 1) AgNP-induced pyroptosis, a non-apoptotic, caspase-1 dependent programmed cell death in differentiated human (THP-1) macrophage cells, and 2) AgNP-induced NLRP3-inflammasome activation and ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD)-pyroptosome formation. Macrophages (LPS primed) were exposed to 10nm and 50nm AgNPs (1, 5, 10, or 25 µg/mL) for 6, 12 and 24 h. Cytotoxicity and pro-inflammatory (IL1b, TNFa) cytokine levels were evaluated by trypan blue dye exclusion and ELISA, respectively. Pyroptosis was confirmed by LDH release, caspase-1 activation (non-apoptotic) and morphological evaluation of nuclei (non-necrotic). NLRP3-inflammasome activation and ASC-pyroptosome formation were analyzed by Western immunoblot. Results showed a) phagocytized 10nm and 50nm AgNP induced significant increases in IL1b (4-fold) and TNFa (5-fold) secretion (≥ 5 µg/ml; 10nm>50nm), b) activation of NLRP3-inflammasome, ASC-pyroptosome formation and pyroptosis after 6 h post-exposure (≥ 5 µg/ml; 10nm>50nm) evidenced by increased caspase-1 activity (3-fold), and c) silencing expression of NLRP-3 and ASC by respective siRNAs and inhibiting caspase-1 activation by chemical inhibition (VX765) reduced AgNP (5, 10 µg/ml)-induced IL-1b production and LDH release. In conclusion, we report a novel mechanism of caspase-1 dependent activation of pro-inflammatory signaling in AgNP-treated macrophages. Specifically, a) phagocytized AgNP increased production of pro-inflammatory cytokines and pyroptosis in human THP-1 macrophages, and b) AgNP induced pyroptosis was mediated by NLRP3-inflammasome dependent caspase-1 activation. These findings a) suggest that caspase-1 can serve as a predictive toxicity biomarker for nanomaterials, and b) may contribute to the design of mechanism-based risk assessments and rapid toxicity screening tests for nanomaterials.

2763 Carbon Nanodots Interference with Lactate Dehydrogenase Assay in Human Monocyte THP-1 Cells

P. C. Wright[2], H. Qin[3], M. M. Choi[3], N. H. Chiu[2] and Z. Jia[2]. [1]*University of North Carolina at Greensboro, Greensboro, NC;* [2]*Biology, University of North Carolina at Greensboro, Greensboro, NC and* [3]*Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong.*

Carbon nanodots (CD), a new class of carbon nanomaterials with sizes below 10 nm, have recently attracted wide attention due to their superiority in water solubility, chemical inertness, and resistance to photobleaching. As a result, CD has found important and wide applications in energy, catalysis, biological labeling, bioimaging and drug delivery. On the other hand, due to the lack of available toxicity data, there is a growing concern regarding the potential risks of CD. Hence, accurate assessment of the cytotoxicity of CD has become more important than ever before. The lactate dehydrogenase (LDH) assay is widely used to detect cytotoxicity of various nanoparticles including CD. Many recent studies used LDH assay to study the CD toxicity in various cells. However, these studies failed to further examine whether the CD were interfering with the LDH assay which would alter their findings. Thus, this study investigated the possible interference of carbon nanodots on the LDH assay in human monocyte THP-1 cells. Monocytes are known to be involved in inflammable vascular diseases, and have been suggested to be the targets for CD exposure. In this study, the cytotoxicity of CD in concentrations ranging from 0.075 to 0.60 mg/mL, was determined by using the LDH assay. To validate the results of LDH assay, the cell counting method with trypan blue staining was used. With 24 hours incubation time, the cell viability of THP-1 was significantly decreased according to the trypan blue staining method. Whereas, in the LDH assay, the CD was found to interfere in a dose-dependent manner with the NADH absorbance measurements at 340 nm. In summary, this study represents the first report on the negative interference of CD on LDH assay, and caution should be observed when evaluating the cytotoxicity of CD.

2764 Effect of Acute and Chronic Silver Nanoparticle (AgNP) Exposure on Osteogenic Differentiation of Human Bone Marrow Stromal Cells (hBMSCs)

A. Nguyen[2], R. Patel[1], B. J. Casey[3], P. L. Goering[3], R. Narayan[2] and G. Kumar[3]. [1]*UMBC, Baltimore, MD;* [2]*UNC/NCstate, Raleigh, NC and* [3]*US FDA, Silver Spring, MD.*

In vitro toxicology evaluations utilizing human stem cell models represent attractive alternatives to conventional animal models, which are not always predictive of human responses. Exposure to nanoparticles released from medical devices may alter the characteristic expression of stem cell surface markers, potentially changing their unique stem cell niche. The aim of this study was to 1) determine non-cytotoxic AgNP concentrations, 2) establish a base level of CD105, CD44, CD73, and CD90 cell surface marker expression in hBMSCs, 3) evaluate changes in surface marker expression under different exposure conditions with varying AgNP concentrations, and 4) observe and quantify phenotypic changes associated with AgNP uptake. Non-cytotoxic concentrations of 10nm, PVP-coated AgNP (≤ 10 µg/mL, MTT assay) were used to evaluate AgNP induced changes in osteogenic differentiation of hBMSCs. Monolayers of cells were treated with 0, 1, 5, or 10 µg/mL in presence or absence of osteogenic differentiation supplements (OS) and in three different exposure scenarios: single, once per week, or continuous exposure. The base level surface marker expression was assessed using fluorescently-labeled antibodies and quantified using flow cytometry. Cell proliferation, surface antigen expression, alkaline phosphatase (ALP) expression and calcium mineral deposition were assessed for each treatment scenario for 1, 7, 14 and 21 days. Untreated hBMSCs co-expressed CD44, CD90, CD105, and CD73 surface markers and were neg-

ative for hematopoietic markers indicating near-pure populations. For single exposure, hBMSCs exposed to low AgNP concentrations (1 ug/mL) in the presence of OS showed a time-dependent, biphasic response in ALP expression and increased calcium mineral deposition. For continual exposure, DNA assay showed that hBMSCs in presence of OS showed low viability (> 98% cell death) at higher AgNP concentrations (5 and 10 ug/ml). Thus, non-cytotoxic AgNP exposure can influence hBMSCs differentiation.

2765 *In Vitro* Dosimetry of Ag Nanoparticles: ICP-MS, Flow Cytometry and the *In Vitro* Sedimentation, Diffusion and Dosimetry Model (ISDD)

J. N. Ortenzio[1,2], L. L. Degn[3], J. R. McGee[3], R. M. Zucker[3] and W. K. Boyes[3]. [1]*Duke University, Durham, NC;* [2]*Oak Ridge Institute for Science and Education, Durham, NC and* [3]*ORD/NHEERL/TAD/NB, US EPA, Research Triangle Park, NC.*

Interpretation of *in vitro* nanomaterial testing begins with quantifying the cellular dose. Given the typically heterogeneous nature of nanomaterial suspensions, the amount of nanomaterial delivered to adherent cells cannot be assumed to be the same as the concentration added to the culture (the applied dose). Here, we compare absorbed dose as measured by inductively-coupled plasma mass spectrometry (ICP-MS) and delivered dose as estimated by an *in vitro* sedimentation, diffusion and dosimetry model (ISDD) (1,2). A retinal pigment epithelial cell line (APRE-19) was exposed to 20 or 75 nm citrate-coated silver nanoparticles (AgNP) for 24 hr. Comparing 20 and 75 nm AgNP, the larger particles deposited on or absorbed by the cells amounted to a greater mass, but fewer particles and smaller surface area. Over 24 hours, the ISDD model predicted a delivered dose of 20 and 75 nm AgNP as 26 and 34 % of applied dose, respectively, whereas ICP-MS measurement indicated internalization of 12 and 30 % of a 10 µg/mL applied dose. Agreement between ISDD and measured total Ag was greater for 75 nm than for 20 nm NPs. This size dependency may reflect differential dissolution of Ag from the NPs that was not accounted for in this version of ISDD or could also indicate that the Ag deposited on the cell layer was not entirely absorbed by the cells. Another concern is the shortage of time and cost efficient techniques for quantifying cellular dose. Previously, we showed that cells absorbed AgNP in a dose dependent manner between 1 µg/mL and 30 µg/mL and could be detected by light scatter using a flow cytometer (3). In this study, flow cytometry side scatter was linearly related to Ag mass measured by ICP-MS, and to calculations of internalized NP number and surface area. Results support use of flow cytometry for rapid and inexpensive measure of cellular dose if pre-calibrated for NP size and composition. This abstract does not reflect EPA policy. 1 Hinderliter et al. Particle and Fibre Toxicology 2010, 7:36 2 DeLoid et al. Nature Communications 2014, 5:3514 3 Zucker, et al. Cytometry A 2013, 83:962

2766 Impact of the Protein Corona on the Blood Compatibility Profile of Gold Nanoparticles

A. Sasidharan and N. A. Monteiro-Riviere. *Nanotechnology Innovation Center of Kansas State, Kansas State University, Manhattan, KS.*

Gold nanoparticles (AuNP) have been proposed for a multitude of biomedical applications, but there is a lack of information on how the protein corona forms over the AuNP surface and how it could influence hematotoxicity. AuNP of 40 and 80nm were exposed to pooled human plasma (n=5) for 1 h to study the evolution of the protein corona using differential centrifugal sedimentation (DCS), dynamic light scattering (DLS) and nanoparticle tracking analysis. To predict whether the adsorbed protein corona could alter the native hemotoxicity profile of AuNP, a battery of common blood toxicity assays were conducted including RBC aggregation, hemolysis, platelet activation, platelet aggregation, complement activation, plasma coagulation, cytokine induction, lymphocyte proliferation and immunosuppression. These results revealed that the protein corona over the AuNP surface significantly influenced the interaction of AuNP with human blood components, and mitigated the harmful effects even at relatively high concentrations of 100 µg/ml. In contrast to the bare particles, the human plasma protein coated AuNP exhibited good compatibility with red blood cells, platelets, lymphocytes and plasma coagulation pathways. Interestingly, irrespective of size or surface functionalization, human plasma treated AuNP did not activate the complement system or cause any immune cell activation or immunosuppression up to 100 µg/ml at 72 h of incubation. This study provides further understanding of the effects of the protein corona on AuNP interaction with human blood components, which has implications in the safe usage of these nanomaterials for future use in biomedical applications.

2767 The Protein Corona Alters Silver Nanoparticle-Induced Endothelial Cell Endoplasmic Stress

I. Persaud, J. H. Shannahan and J. M. Brown. *Pharmaceutical Sciences, University of Colorado, Denver, CO.* Sponsor: J. Brown.

Silver nanoparticles(AgNPs) are widely utilized in consumer and medical products due to their antimicrobial properties. This has resulted in their increased introduction into biological systems. Prior research has demonstrated cells exposed to AgNPs exhibit acute toxicity and induction of endoplasmic reticulum(ER) stress. ER stress is the result of misfolded proteins and ultimately will result in the induction of apoptosis. AgNPs have been shown to associate with a variety of proteins forming a protein corona, which has been shown to alter NPs properties, cellular interactions, and toxicity. Currently there is a lack in understanding of how the protein corona can modify cellular toxicity through the ER stress pathway. Our study investigates the ER stress cellular response after exposure to 20 and 100nm AgNPs without or with coronas consisting of albumin, high-density lipoprotein(HDL), or fetal bovine serum proteins (complex corona). Using cellular assays with rat aortic endothelial cells(RAEC), AgNPs without or with protein coronas were investigated for the effects on viability, ER stress gene expression, protein expression, and alterations in coronal characteristics following internalization. Exposure to 20nm AgNPs (20µg/mL,3hr) resulted in reduced cell viability while exposure to 100nm AgNPs caused no changes. Analysis of mRNA levels by PCR demonstrated increased ER stress related gene expression of endothelial cells exposed to HDL coated AgNP compared to uncoated AgNPs. Our results indicated an increase in pIRE/IREα for 20nm and 100nm AgNPs without a protein corona compared to controls and uncoated NPs. HDL coated AgNPs exhibited higher pIRE/IREα levels compared to albumin and FBS coating. Hyperspectral analysis and TEM indicated internalization of both 20 and 100 nm AgNP. Subcelluar localization from TEM analysis determined that 20nm were mainly located in multi-vesicular bodies whereas 100nm were located in lysosomes. Hyperspectral analysis indicated the presence of proteins on all AgNPs after internalization. The presence of protein on the NPs, and dissociation after internalization may play a role in ER stress through an unfolded protein response due to altered protein structures. This increase in ER stress may ultimately result in apoptosis. This data will allow for the understanding of protein interactions with NPs and offer insights to engineer safe and effective NPs. This work was supported by NIEHS (R15ES022766).

2768 Evidence for Involvement of the NLRP3 Inflammasome in the Dose-Dependent Oxidative DNA Damage Induced by PVP- and BPEI-coated Silver Nanoparticles in THP-1 Monocytes

W. Ding and L. Lyn-Cook. *Division of Genetic and Molecular Toxicology, US FDA/National Center for Toxicological Research, Jefferson, AR.*

Various types of coated silver nanoparticles (AgNPs) have been exploited in commercial applications; however, the effects of these AgNPs on human health are largely unknown. Previous *in vivo* studies indicate that AgNPs, whether administered by inhalation, ingestion or intra-peritoneal injection, subsequently are detected in blood, mostly in peripheral blood monocytes. In the current study, we compared the oxidative DNA damage induced by negatively charged polyvinyl pyrolidone (PVP)-coated 10-nm AgNPs (AgNP10s) and positively charged branched polyethyleneimine (BPEI)-coated AgNP10s inTHP-1 monocytes. Oxidative DNA damage was monitored by the human 8-oxo-guanine DNA glycosylase (hOGG1)- and endonucleaseIII (EndoIII)-modified alkaline comet assays. THP-1 monocytes were cultured with PVP-coated AgNP10s and BPEI-coated AgNP10s for 4 hr; the silver ion (Ag+) was used as a positive control. Both PVP- and BPEI-coated AgNP10 induced dose-dependent increases in oxidative DNA damage, with a five-fold-higher dose of PVP-coated AgNP10s than BPEI-coated AgNP10s needed to induce a statistically significant increase. To investigate the role of the NLRP-3 (NOD-like receptor family, pyrin domain containing 3) inflammasome in AgNP-induced oxidative stress, THP1-defNLRP3 cells, THP-1 monocytes with reduced NLRP3 activity, were treated with the same concentrations of PVP- and BPEI-coated AgNP10s. Much less oxidative DNA damage was detected in THP1-defNLRP3 cells. Taken together, our observations suggest that BPEI-coated AgNP10s induce more oxidative DNA damage in THP-1 monocytes than PVP-coated AgNP10s and that the AgNP10-induced oxidative DNA damage was mediated by the involvement of NLRP3 inflammasome in THP-1 monocytes.

2769 Assessment of Nanoparticle Toxicity in the Context of Virus Infection

S. Agnihothram, L. Mullis, Y. Zhang, T. A. Townsend, M. Manjanatha and M. Azevedo. *Microbiology, National Center for Toxicological Research, Jefferson, AR.*

Titanium dioxide nanoparticles (TiO2) are increasingly used in the manufacturing of dietary supplements and cosmetics. Accumulation of nanoparticles in the intestine has been reported as a result of consumption of nanoparticles used as food additives, which could impart toxicity to the host by altering immune response and cellular metabolism. Mammalian intestine serves as a resourceful host for a variety of enteroviruses, and therefore the presence of viral toxins could exacerbate host response and immune toxicity from nanoparticles. To address this issue, we utilized murine norovirus infection of RAW 264.7 macrophages as a model system to investigate the toxicity of nanoparticles in the context of viral infection. The size, distribution, surface charge, stability, purity and endotoxin levels of the nanoparticles were determined prior to the study. RAW 264.7 cells were treated with TiO2 in anatase and rutile forms at concentrations of 20ug/ml and 2ug/ml for 3h and then infected with Murine norovirus at a multiplicity of infection (MOI) of 5, 0.5 and 0.05 particles. Plaque assay and real-time RT-PCR results indicated that TiO2 nanoparticles inhibited, or did not alter, norovirus replication even at MOI 5. Interestingly, pretreatment of cells with anatase and rutile forms increased viral replication up to seven-fold when infected at lower MOI. While preincubation of TiO2nanoparticles did not impact cytopathic effects to RAW macrophages, virus-induced cytopathicity was not prevented by nanoparticles. Cytokine analysis indicated that there was an increase in Tumor Necrosis Factor Alpha and Interleukin (IL)-12 due to the presence of nanoparticles during virus infection, while IL-6 was found to be reduced or unaltered. Comet assays and further gene expression profiling are underway to identify the genotoxic potential of nanoparticle accumulation in the context of viral infection. This study will elucidate safety and toxicity hazard profile prediction of nanoparticles exposed to the gastrointestinal tract in individuals infected with enteric viruses.

2770 Cytotoxic Effects Induced by Titanium Dioxide Aerosol Nanoparticles

J. J. Kim. *Sungkyunkwan University, Seoul, Korea, Democratic People's Republic of.* Sponsor: D. Y. Shin.

Titanium dioxide (TiO2) has widely been used such as cosmetics and industrials. However, investigations about toxic effects of TiO2 have been discovered. In particular, toxicity of TiO2 under aerosol exposure needs to be more demonstrated. The aim of this study is to elucidate the cytotoxicity effects of TiO2 aerosol nanoparticles by using the Cultex system. Before exposure, the calibration for the airway flow in cultex system was performed. The size distribution of TiO2 was measured by scanning mobility particle sizer (SMPS). Inductively coupled plasma mass spectrometry (ICP-MS) was used for the measuring a deposition efficiency. In addition, MTS assay was implicated as a cytotoxicity test. The initial input of air flow is total 2.3 liter per minute (LPM) in cultex system. In that amount of it, air flow of sampling pump and CO2 part is setted at 0.9 LPM, 0.05 LPM, respectively. Our air flow of total, sampling pump and CO2 part was 2.33 ± 0.009 LPM, 0.90 ± 0.001 LPM, 0.051 ± 0.001 LPM, respectively, which satisfied with all the set up value. Moreover, the average size was 59 ± 2.2 nm by SMPS. When exposed the TiO2 aerosol nanoparticles, the deposition rate was below 10% demonstrated by ICP-MS. Furthermore, cell viability was not diminished below 80% in MTS. After all, no cytotoxicity effect of TiO2 was observed at the aerosol exposure.

2771 Toxicity of Nano- and Ionic Silver to Embryonic Stem Cells - A Comparative Toxicogenomic Study

X. Gao, V. D. Topping, Z. Keltner, R. L. Sprando and J. J. Yourick. *Office of Applied Research and Safety Assessment, US FDA, Laurel, MD.*

The widespread application of silver nanoparticles (AgNPs) and silver-containing products has raised public safety concerns about their adverse effects on human health and the environment. To date, *in vitro* toxic effects of AgNPs and ionic silver (Ag+) on many somatic cell types are well established. However, only limited studies have been conducted in embryonic stem cells (ESCs) to evaluate their potential of developmental toxicity. In this study, we characterized transcriptomic changes induced by 5.0 µg/ml AgNPs during spontaneous differentiation of mouse ESCs, and compared to those induced by Ag+ under otherwise identical conditions. After 24 h exposure, 101 differentially expressed genes (DEGs) were identified in AgNP-treated cells, whereas considerably more (400) genes responded to Ag+. Despite the large differences in the numbers of DEGs, functional annotation and pathway analysis of the regulated genes revealed overall similarities between AgNPs and Ag+. In both cases, most of the functions and pathways impacted fell into two major categories, embryonic development and metabolism. Nevertheless, a number of canonical pathways related to cancer were found for Ag+ but not for AgNPs. Conversely, it was noted that several members of the heat shock protein and the metallothionein families were upregulated by AgNPs but not Ag+, suggesting specific oxidative stress effect of AgNPs in ESCs. Taking together, these results suggest that both AgNPs and Ag+ have the potential to cause developmental toxicities, and that although transcriptomic responses to AgNPs and Ag+ were substantially similar, AgNPs may exert specific effects on ESCs due to their nanosized particulate form.

2772 Assessing Genotoxicity of Iron Oxide Nanoparticles within an *In Vitro* Liver 3D Model

J. de Oliveira Mallia[1,2], N. Singh[2], S. Bohic[1], U.-K. Shah[2], G. Jenkins[2] and S. Doak[2]. [1]*ESRF INSERM U-836 Equipe 6, Grenoble Institut des Neurosciences, Grenoble, France and* [2]*In Vitro Toxicology Group, Institute of Life Sciences, Swansea, United Kingdom.* Sponsor: G. Johnson.

Nanomaterials have demonstrated their applicability in various fields. Risk evaluations are still required for their safe application in order to assess and understand possible toxicity effects. The hepatic system is where these effects might possibly be observed as it is highly involved in the clearance of nanomaterials. The liver 3D *in vitro* model used was developed in order to evaluate potential nanomaterial genotoxic effects. Initially, growth and metabolic characteristics of the model were evaluated over a seven day period. The growth parameters investigated involved surface area measurements and cell viability using Trypan blue staining. Albumin and aspartate transaminase secretion, and CYP1A2 expression were the metabolic liver functions investigated. Optimal growth and metabolic characteristics were achieved after four days. Genotoxic assessments for dextran coated superparamagnetic iron oxide (Fe2O3) nanoparticles were then conducted on the fourth day. Micronucleus frequencies were investigated using a cytokinesis block micronucleus assay that showed significant ($P<0.05$) increases in micronucleus formation. Significant differences were not observed for the replicative indexes determined. Cell viabilities over 90% were retained after 48 hours of exposure. Scanning electron microscopy was used to observe the surface distribution of these nanoparticles. Additionally, synchrotron X-ray fluorescence was employed to investigate the distribution of these nanoparticles within the model. The 3D spheroid models represent an improved and more realistic test environment to conduct *in vitro* genotoxicity studies than standard 2D systems. Its versatility also enables the application of various methods involved in nanomaterial studies.

2773 Genotoxicity of Pristine, Heat-Treated, and Nitrogen-Doped Multi-Walled Carbon Nanotubes at Occupationally Relevant Doses

K. Siegrist[1], S. Reynolds[1], R. Mercer[1], D. Porter[1], D. Lowry[1], L. Cena[1], M. Kashon[1], J. Salisbury[2], J. Mastovich[4], K. Bunker[4], M. Sparrow[4], S. Tsuruoka[5], M. Endo[5], M. Terrones[3], M. McCawley[6] and L. Sargent[1]. [1]*CDC/NIOSH, Morgantown, WV;* [2]*Mayo Clinic, Rochester, MN;* [3]*Pennsylvania State University, State College, PA;* [4]*RJ Lee Group, Monroeville, PA;* [5]*Shinshu University, Nagano, Japan and* [6]*West Virginia University, Morgantown, WV.*

The unique physiochemical properties of multi-walled carbon nanotubes (MW) make respiratory exposures likely in workers. Previously, *in vitro* exposure to MW has led to cell cycle disruption, chromosome errors, mitotic aberrations, and increased clonal growth at occupationally relevant doses. Combining the effects seen *in vitro* with the potential for lung deposition in the workplace, MW should be considered as a potential health hazard. Altering the physiochemical properties of MW has been shown to reduce toxicity. Nitrogen-doped MWCNT (NDMW) material is less inflammatory than pristine MW (PMW). PMW exposed to extremely high temperatures (HTMW) removes impurities and reduces their bioreactivity in acellular systems. To investigate genotoxicity of NDMW and HTMW compared to PMW at an occupationally relevant dose, we used two cell types, an immortalized human lung epithelial cell BEAS-2B and primary lung epithelial cell SAEC. All MW were necrotic in both cell types at the 24 µg/cm2 dose. There was no effect on the cell cycle in BEAS-2B cells. All MW induced a significant G0/G1 phase block in SAEC cells after 24 hour exposure to 24 µg/cm2 and a significant G1/S phase block after 72 hour exposure to 2.4 µg/cm2. Clonal growth in SAEC cells was inhibited by PMW at all doses, HTMW at 24 µg/cm2 and

NDMW at 2.4 and 24 µg/cm2. By contrast, number and size of colonies was increased by 0.024 and 0.24 µg/cm2 HTMW and NDMW and 2.4 µg/cm2 HTMW. Significant increases in mitotic aberrations, predominantly monopolar, were observed by exposure to all MW for 24 hours. All MW were found to penetrate the nucleus and associate with the DNA, mitotic spindle and centrosomes. Nuclear penetrations were greatest for the PMW followed by HTMW and NDMW, respectively. Significantly increased fragmentation of the centrosome and centromere were found in response to all MW exposure at all doses indicating a possible mechanism of genotoxicity, however a lower limit was not apparent. A dose-dependent increase in aneuploidy was observed from exposure to all MWs. These data indicate that altering the physiochemical properties of MWs may not reduce their genotoxic effect.

2774 Size and Crystal Structure Dependent Inhibition of Human Mesenchymal Stem Cell Adipogenic Differentiation by Nanoscale Titanium Dioxide

J. Yao[1,3], Y. Jones[3], W. Monroe[3], J. Collins[3], P. Howard[3], A. K. Patri[3] and Y. Zhang[3]. *[1]US FDA Commissioner's Fellowship Program, Jefferson, AR; [2]NCTR/ORA Nanotechnology Core Facility, Jefferson, AR and [3]Office of Scientific Coordination, National Center for Toxicological Research, NCTR/ORA Nanotechnology Core Facility, Jefferson, AR.*

Titanium dioxide (TiO2) has been used in a broad spectrum of consumer products, including food, cosmetics and various medical products. Increased use of nano-scale TiO2 in recent years has raised concern regarding their safety. In the current study, we characterized TiO2 nanoparticles with different crystal structures and particle sizes through electro microscopy, diffraction and scattering techniques. . Subsequently, we determined the impact of TiO2 nanoparticles on cell viability using LDH, ATP assays, and the adipogenic differentiation capacity using Oil red O Staining assay in human mesenchymal stem cells (hMSCs). We further investigated whether the impact of TiO2 nanoparticles was associated with specific particle size and/or crystal structure. Data revealed that TiO2 nanoparticles exhibited minimal acute (up to 72 hours exposure) cytotoxicity in hMSCs. There was a size- and crystal structure- dependent inhibition of hMSC adipogenic differentiation (21 days) by TiO2 nanoparticles. Cellular uptake and media "stripping" studies indicated that the inhibition of hMSC adipogenesis was likely due to direct cellular response to TiO2 nanoparticles instead of a "charcoal-stripping" effect of TiO2 leading to depleted growth factors in the culture media. Additional exploratory gene expression array analyses suggested that TiO2 nanoparticles inhibit hMSC adipogenesis by down-regulating key genes involved in adipogenesis promotion, including FGF2, IRS1, CEBPA, CEBPB, and ACACB, etc. Findings from this study indicate that TiO2 nanoparticles, while exhibiting minimal acute cytotoxicity, may impost long-term impact on hMSC adipogenic differentiation. Future planned studies will reveal the mechanism of TiO2 nanoparticle interaction in stem cell models. Disclaimer: The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

2775 *In Vitro* Toxicity of Tio2 Nanoparticles Immobilized on Clay to Human Hepatic Cells

M. J. Bessa[4], J. J. Reinosa[2], J. F. Fernández[2], M. Bañares[1], J. P. Teixeira[3,4] and C. Costa[3,4]. *[1]Catalytic Spectroscopy Laboratory, Instituto de Catálisis y Petroleoquímica, CSIC, Madrid, Spain; [2]Electroceramic Department, Instituto de Cerámica y Vidrio, CSIC, Madrid, Spain; [3]EPIUnit-Institute of Public Health, University of Porto, Porto, Portugal and [4]Portuguese National Institute of Health, Porto, Portugal.* Sponsor: E. Coskun.

Introduction: Nanotechnology is growing in rapid pace, discovering innovative and attractive nanomaterials for several applications. The fact that nanoparticles induce more damage and are more biologically active when compared to larger micro-sized particles encouraged the emergence of nanoarchitectonics. The immobilization of nanoparticles on the surface of inorganic or organic supports results in the creation of nanocomposites that combine the best properties of both components. Although it has been stated that micro-sized particles induce less toxicity than the nano-size ones, few studies have been made regarding the *in vitro* characterization of cellular responses to this type of materials. Objective: Evaluate *in vitro* toxicity of TiO2 nanoparticles immobilized on clay (C-TiO2) in a hepatocellular carcinoma human cell line (HepG2) as well as of its single elements. Materials and Methods: Materials were supplied by the Ceramic for Smart System Group of the Electroceramic Department, Instituto de Ceramica y Vidrio, Madrid, Spain and characterized by scanning electron microscopy for particle morphology and dynamic light scattering for average hydrodynamic size and potential zeta. After characterization, different concentrations and time periods were tested in regards of HepG2 viability, by employing MTT and Alamar Blue assays, and DNA integrity, by using comet assay. Results and Discussion: Results showed that all studied materials were capable to induce hepatocyte cell death in a dose dependent way and for the majority of the studied periods of exposure. Besides that, the HepG2 DNA was also affected after longer periods of exposure to TiO2 NPs, kaolinite and C-TiO2 nanocomposites. Conclusions: Nanocomposites are promising materials for different nanotechnological applications. Notwithstanding, it is of paramount importance to evaluate their potential toxicity. Data obtained suggests that other subtracts must be tested to immobilize TiO2 NPs as kaolinite mineral was found to be both cytotoxic and genotoxic for the studied cell line. Acknowledgements: Financial support from TD1204 MODENA COST Action.

2776 Effects of Zinc Oxide Nanomaterials on the Cellular Responses in THP-1 Cells

A. Miyajima-Tabata, T. Kawakami, K. Komoriya, R. Kato, S. Niimi and K. Isama. *National Institute of Health Sciences, Tokyo, Japan.* Sponsor: A. Hirose.

Purpose: The biological effects of nanomaterials are related to the physicochemical properties such as composition, shape, particle size, aggregation state, surface area and surface charge. An *in vitro* cellular toxicological study using well-characterized nanomaterials is conducted for evaluation of the biological effects of nanomaterials. In this study, we examined the effects of zinc oxide (ZnO) nanomaterials on the cytotoxicity of THP-1 cells and the expression of CD54 and CD86. Methods: The size distribution and the zeta potential of ZnO nanomaterials were measured by dynamic light scattering. The cellular cytotoxicity and the expression of CD54 and CD86, skin sensitization marker, were measured by cellular ATP method and flow cytometry, respectively. Results and Discussion: The primary particle size and hydrodynamic diameter of ZnO nanomaterials in distilled water suspension were <35 nm and 66 nm (Sigma-Aldrich), and 40 nm and 165 nm (NanoTeK Alfa Aesar). The zeta potentials of ZnO nanomaterials in distilled water suspension were positive (44.9 mV, Sigma) and negative (-7.5 mV, Alfa). The cytotoxicity by ZnO (Sigma) was stronger than that by ZnO (Alfa). ZnO nanomaterials increased the CD54 in a dose-dependent manner, but did not affect the expression of CD86. The CD54 relative fluorescence intensity (RFI) after treatment with 50 µg/mL of ZnO for 24h were 2007 % (Sigma) and 1207 % (Alfa). In conclusion, ZnO nanomaterials showed the cellular cytotoxicity and increased the expression of CD54. The extent of these effects caused by ZnO (Sigma) was higher than that by ZnO (Alfa). The differences of the cellular responses may due to the differences of physicochemical properties between ZnO (Sigma) and ZnO (Alfa). Further analysis at the molecular-level would help the better understanding of the relation between biological effects and the physicochemical properties of nanomaterials.

2777 Induction of the Epithelial-Mesenchymal Transition by Zinc Oxide Nanoparticles

J. J. Kim. *Sungkyunkwan University, Seoul, Korea, Democratic People's Republic of.* Sponsor: D. Y. Shin.

Zinc oxide (ZnO) nanoparticles are one of the promising materials applied in the various kinds of the commercial products such as sunscreens. At the same time, there are growing concerns about unintended toxic effects of ZnO. Many researches on the toxicity of ZnO have been studied, except for the epithelial-to-mesenchymal transition (EMT). EMT is one of the significant multistep processes. Epithelial cells reduce intercellular adhesion and increase cell mobility which is crucial for the cancer metastasis. The aim of our study is to investigate the ZnO-induced EMT in the human alveolar epithelial A549 cells. At first, size distribution of ZnO nanoparticles was observed by dynamic light scattering (DLS). The cytotoxicity test was performed to find the appropriate exposure concentration. Real-time PCR was used for the mRNA expression, related with EMT. In addition, the EMT-associated protein level was measured by western blot. The morphology changes were obtained by the microscopy. The average size of ZnO showed 208 ± 16.10 nm measured by DLS. Cell viability as the cytotoxic effects was decreased in a dose-dependent manner. When A549 cells are exposed to ZnO nanoparticles, mRNA level of EMT-related transcription factor was increased including Snail. Furthermore, the protein levels related with EMT markers were found. In microscopy, ZnO induced morphology changes of A549 to spindle-like elongated shapes. Our data demonstrated that ZnO nanoparticles induced the EMT in A549 cells.

2778 Nanosafety Assessment of TiO2 Nanoparticle-Embedded Cosmeceutical Nanotoxicity on Human Skin and Lung Cells

H. Lee[2], C.-W. Lee[1], H. Kim[2], K. Lee[5] and J. Kim[4]. *[1]Boditech Med Inc., Chuncheon, Korea, Republic of; [2]Detroit R&D, Inc, Detroit, MI; [3]Dept. of Nanotechnology, Detroit R&D, Inc, Detroit, MI; [4]Dept. of Pharmaceutical Sciences, Northeastern University, Boston, MA and [5]Inhalation Toxicology Research Center, Korea Institute of Toxicology, Daejeon, Korea, Republic of.* Sponsor: H. Kim.

The toxicity and the exact subcellular localization of various nanocosmeceuticals need to be critically evaluated before their applications for phototherapy for skin cancer and UV protection. Although many nanomaterials have already been reported to penetrate the cell membrane, and enter the cells, the mechanisms involved in these processes and the exact intracellular identification of nanomaterials or chemically modified nanocosmeceuticals has not been determined. As the surface chemistry plays a key role in interactions of the nanoparticles (NPs) with the cell membrane, it is essential to understand the effect of the surface modifications (which change the size, surface charge, and surface energy of nanomaterials) on their interaction with cells. The effect of the TiO2 nanoparticles modified with palmitoleic, palmitic, stearic and oleic acids [cosmeceutical (CM)-NPs] on human skin fibroblast and lung adenocarcinoma cells was investigated and compared with bare TiO2 nanoparticles (B-NPs) in order to assess the safety of the nanomaterials. Cell toxicity, internalization and intracellular localization of these NPs were measured using NP-exposed cells by a variety of techniques, including electron spectroscopy imaging-transmission electron (ESI-TEM) analysis. Our experiments revealed that both B-NPs and CM-NPs entered the cells after 24 h of exposure. Agglomerates of B-NPs as well as individual B-NPs were observed in large quantities inside human skin and lung cells. Our results demonstrated that the TiO2 nanoparticles modified with fatty acids appeared to be less toxic than B-NPs in both human skin and lung cells.

2779 Evaluating the Cytotoxic Effects of Cellulose Nanocrystals (CNCs) Using Autobioluminescent Yeast and Human Cells

J. Burchett, T. Xu, G. Sayler and S. Ripp. *The Center for Environmental Biotechnology, The University of Tennessee Knoxville, Knoxville, TN.* Sponsor: S. Ripp.

Cellulose nanocrystals (CNCs) are widely used in different industries including pharmaceutical and cosmetic production due to their adept physical and biological properties. Because CNCs are becoming a more prevalent material and have a high potential of being redistributed in the environment, it is important to understand their toxic potentials in biological systems, including organisms of various trophic levels. This study evaluated the cytotoxic effects of CNCs in the lower eukaryotic organism Saccharomyces cerevisiae and human embryonic kidney (HEK293) cells using autobioluminescent yeast and human cell reporters, respectively. The S. cerevisiae and HEK293 reporter cells were engineered to express a synthetic bacterial luciferase operon (luxCDABE) that self-generates all the required substrates for bioluminescent production. As a result, these reporter cells allow for continuous monitoring of the same cell population throughout the period of toxicant exposure, providing a facile means for tracking the temporal dynamics of toxic effects on living cells. When exposed to CNCs at concentrations ranging from 0.001 g/L to 1 g/L, both the yeast and human cells reported time and dose-dependent effects. Exposure to CNCs at 0.001 g/L and 1 g/L reduced bioluminescent output in S. cerevisiae by 5% and 10% compared to untreated control cells 8 hours post-treatment, respectively, and further decreased the signal by 25% and 70% 12 hours post-treatment, respectively. In HEK293 cells, treatment with CNCs at 1 g/L initialized a significant decrease (by 23%) in metabolic activity at 2 days post-treatment, and the bioluminescent output continued to decline to less than 10% compared to untreated controls at 7 days post-treatment. CNCs at 0.001 g/L did not result in significant changes in metabolic activity throughout the entire period of exposure. These results demonstrate the cytotoxic potential for elevated concentrations of CNCs in varying biological systems.

2780 Hydroxyl Radical Generation and Cytotoxicity of Zinc Nanoparticles in RAW 264.7 Cells

A. Morris[4], A. Stefaniak[4], K. Dunnick[2], M. Badding[1] and S. Leonard[4]. *[1]Exponent, Washington, DC; [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC; [3]Health Effects Laboratory Division, CDC/NIOSH, Morgantown, WV and [4]Respiratory Health Division, CDC/NIOSH, Morgantown, WV.*

Handling nanoparticles presents novel hazards to human health, especially when used commercially before possible toxic effects may be evaluated. Zinc nanoparticle use is expanding and exposures are possible during the manufacture of concrete, rubber, food products, sunscreen, and paint. The toxicity of zinc nanoparticles has been investigated but little is known regarding zinc nanowires, a material with properties that make it ideal for solar cells and electronics. In this study, zinc metal nanoparticles (MNP), zinc oxide nanoparticles (NP), zinc oxide micron particles (MP), and zinc oxide nanowires (NW) were comparatively investigated. Potential toxic effects due to inhalation exposures were studied using RAW 264.7 mouse macrophage cells. Shape, diameter, and percentage of zinc in the sample were also characterized. A CellTiter-fluor assay was used to determine cell viability and a CytoTox assay was used to determine lactate dehydrogenase (LDH) release following 4 h and 24 h exposures for three different particle doses (10, 25, and 50 µg/ml) to assess cytotoxicity of the various particles. Electron Paramagnetic Resonance (EPR) was used to determine free radical production in both acellular and cellular experiments. There was a trend towards decreased viability at 4 h with the highest dose and at 24 h with the middle dose, but changes were only significant at 24 h with 50µg/ml for all particle types. LDH levels in cell culture media were significantly increased with MNP treatment at 50µg/ml for 4 h. After 24 h, all particles at 25 and 50 µg/ml caused significant LDH release. EPR results indicated that MNP stimulated significantly greater hydroxyl radical (·OH) production than NP, MP, and NW upon reaction with H2O2 and in the presence of RAW cells. Our results demonstrate that while MNP stimulated the most ·OH production, all of the zinc particles decreased cell viability, suggesting multiple mechanisms for zinc nanoparticle cytotoxicity.

2781 ZnO Nanoparticle Ingestion Alters Intestinal Epithelial Function

F. Moreno Olivas[1], E. Tako[2] and G. Mahler[1]. *[1]Biomedical Engineering, Binghamton University, Binghamton, NY and [2]Plant, Soil and Nutrition Laboratory, Agricultural Research Services, US Department of Agriculture, Ithaca, NY.*

Zinc oxide nanoparticles (ZnO NPs) are used to make cans of sulfur producing foods, which provides a risk for ZnO NP ingestion. Canned foods were analyzed for Zn content with ICP-MS and TEM. These results showed that a serving of canned food contains approximately 1.4x10^15 NPs per meal. Doses used for experiments were determined by dividing this amount by the surface area of the small intestine or the surface area of the jejunum for a low (7.12x10^8 particles/cm2) and high dose (1.78x10^9 particles/cm2), respectively, and an intermediate dose (1x10^9 particles/cm2) was also investigated. 10 nm particles (Mellorium Technologies, Inc.) were characterized using dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). An *in vitro* model of the GI tract containing Caco-2 and HT29-MTX cell line co-cultures and a simulated digestion was used for ZnO NP exposure studies. Characterization of the ZnO nanoparticles with TEM showed that placing the particles in cell medium resulted in smaller aggregates (90-93 nm) than in water (300-450nm), DLS and NTA showed aggregate sizes of 130 and 178 nm in water, respectively. *In vitro* digestion was shown to partially dissolve the NPs. For nanoparticle exposure experiments, the three doses were used for both acute (4 hours) and chronic (5 days) time periods. After exposure to ZnO NPs, iron, zinc and copper cell uptake and transport were measured by adding stable isotopes (58Fe, 63Cu, and 67Zn) to the digest and cell medium, collecting the medium, and analyzing the isotope content with ICP-MS. Glucose transport was modeled using the fluorescent glucose analog 2-NBDG. Cell viability was measured with a MTT assay, oxidative stress was measured using CellROX® (Life Technologies) reagent, nutrient transporter gene expression was determined using RT-PCR, and tight junction functionality was assessed using transepithelial resistance and immunocytochemistry for the tight junction protein occludin. Following both acute and chronic exposure, cell viability decreased, which may be the result of the Zn2+ ions that dissolve in the cell medium or digest. ZnO NP exposure increased ROS formation and decreased nutrient transport and uptake and occludin expression in the intestinal epithelial cells as well as influenced in the expression of transporter genes, suggesting that ZnO ingestion may result in significant metabolic effects.

2782 An Evaluation of Genotoxicity from As-Produced and Post-Production Modification of Multi-Walled Carbon Nanotubes

K. Siegrist[1], S. Reynolds[1], C. Mitchell[3], D. Lowry[1], M. Kashon[1], L. Bishop[1], A. Erdely[1], J. Bonner[2], G. Parsons[2], C. McClure[2] and L. Sargent[1]. *[1]CDC/NIOSH, Morgantown, WV; [2]North Carolina State University, Raleigh, NC and [3]West Virginia University, Morgantown, WV.*

Companies synthesize or purchase bulk as-produced multi-walled carbon nanotubes (AP-MW) and apply coatings such as polymers (PC-MW) or aluminum oxide (AL-MW). The aim of this study was to measure the genotoxic response of three AP-MW (1, 2 & 3) and their coated counterparts (PC-MW 1 & 2 and AL-MW 3) to determine the effect of coating. Genotoxicity was measured via micronuclei assay with pancentromere staining and two-color cell cycle analysis. Pancentromere staining determined whether the DNA damage is clastogenic (chromosome breakage) or aneugenic (whole chromosome gain or loss). Immortalized human lung epithelial cells, BEAS-2B, were exposed to all MW for 24 hours. Significant necrosis was measured for all but AP-MW1 at the highest dose, 24 µg/cm2. Exposure to the lowest and most occupationally-relevant dose, 0.024 µg/cm2, of AL-MW was also significantly necrotic. Tunel assay of cells exposed to 2.4 µg/cm2 MW for 24 hours showed no evidence of apoptosis. All but PC-MW2 produced increased amounts of micronuclei after exposure to 24 µg/cm2 for 24 hours; AP-MW2 and AP-MW3 had greater micronuclei than their coated counterparts. All MW produced increased amounts of micronuclei after exposure to 2.4 µg/cm2 for 24 hours, however differences from the effect of coating cannot be detected at this time. Pancentromere staining indicated AL-MW to be a clastogen at the 2.4 µg/cm2 dose, while all other MW induced a combination of clastogenic and aneugenic effects. Preliminary two-color cell cycle analysis showed no evidence of cell cycle disruption after 24 hour treatment to 2.4 µg/cm2 MW. In order to determine the effect of coating on the genotoxic response, further analysis of the mitotic spindle and separation of genetic material leading to aneuploidy is required. However, this preliminary evaluation indicates that human lung epithelial cells exposed to AP-MW and their coated counterparts produces a genotoxic response.

2783 Nano-Ferric Oxide Induced Neoplastic-Like Transformation in a Human Primary Cell Model: Iron Homeostasis Disruption?

T. A. Stueckle[3], D. C. Davidson[3], R. Derk[3], P. Demokritou[1], T. Kornberg[3], D. Schwegler-Berry[3] and L. Wang[3]. *[1]Harvard School of Public Health, Boston, MA; [2]National Institute for Occupational Safety and Health, Morgantown, WV and [3]Allergy and Clinical Immunology Branch, National Institute for Occupational Safety and Health, Morgantown, WV.*

As incorporation of engineered nanomaterials (ENMs) into new technologies rise, the potential for long-term, low dose inhalation exposures is expected with largely unknown adverse outcomes to human health. Past ENM toxicity assessment has focused on acute and relatively short term sub-chronic exposures associated with inflammation and fibrosis, with relatively little attention paid to ENM-associated tumorigenesis. The current study evaluated the use of a human primary small airway epithelial cell (pSAEC) model to serve as a Tier I neoplastic transformation screening model for proposed ENM tiered risk assessment. Low passage pSAECs were continuously exposed *in vitro* to 0.6 µg/cm2 of nano-sized cerium oxide (CeO2) or ferric oxide (Fe2O3) for 6 and 10 weeks. Multi-walled carbon nanotube (MWCNT Mitsui 7; 0.06 µg/cm2), with known transformation and lung cancer promotion potential, served as a positive control while saline or dispersant exposed cells served as passage controls. At each time point, exposed cells were evaluated for several cancer hallmarks to evaluate neoplastic transformation potential. At 10 weeks, Fe2O3-exposed cells displayed significant enhanced proliferation, invasion, soft agar colony formation and colony forming unit ability suggesting a neoplastic-like transformation. MWCNT-exposed cells exhibited increased colony formation ability while nCeO2 was negative in all assays except proliferation. Next, Fe2O3 and MWCNT soft agar colonies were isolated and placed in culture to evaluate persistence and longevity of the transformed phenotype. All isolated clones, along with the 10 week Fe2O3-exposed cells, maintained their transformed phenotypes, even after repeated (12-30) passages and freezing. Further studies using fluorescent imaging and protein expression analysis suggested that potential disruption to iron homeostasis gave rise to increased intracellular iron and increased ROS production which is well-known to cause oxidative damage and promote cell transformation. Sub-chronic *in vitro* exposures with cancer hallmark screening using human primary cell models holds promise as a Tier I screening model for ENM-associated tumorigenesis.

2784 Vacuole Formation and Caspase-Dependent Apoptosis in Human Monocytes Caused by Exposure to Titanate Nanosheet

Y. Nishimura, D. Yoshioka, S. Lee, N. Kumagai-Takei, H. Matsuzaki, K. Yoshitome and T. Otsuki. *Department of Hygiene, Kawasaki Medical School, Kurashiki, Japan; Department of Natural Sciences, Kawasaki Medical School, Kurashiki, Japan.*

Toxicities of various kinds of nano-sized materials have been studied recently, which have demonstrated inflammatory response and tumor development caused by exposure to those. Titanate nanosheet (TNS) is a representative sheet-like nano-sized material with depth of about 1 nano meter, and has been developed as a material for UV- or corrosive-resistant films, dielectric thin films and catalysts. Therefore, we examined effects of exposure to TNS on human peripheral blood mononuclear cells (PBMCs) or isolated CD14+ monocytes. TNS was synthesized from Ti(O-i-Pr)4 and NEt4OH by a method of liquid-phase synthesis. PBMCs were cultured with TNS and assayed for apoptosis by flow cytometry and observed for morphological changes. PBMCs showed annexin V+ PI- apoptotic cells after 7 days of culture with TNS at more than 2 µg/ml, in contrast to early apoptosis after 2 days of culture with asbestos. The addition of Q-VD-OPh, pan-caspase inhibitor suppressed apoptosis caused by exposure to TNS. TNS-exposed PBMCs showed vacuole-forming cells, which were positive for CD14. Isolated CD14+ monocytes generated vacuoles until after 1 day of the culture with TNS, and vacuoles enlarged by the day. The observation by transmission electron microscopy showed the existence of nano-sized materials with TNS-like shape inside vacuoles. TNS exposure affected endosome structures, visualized by pre-incubation with fluorescence-labeled dextran, and vacuoles in those monocytes included fluorescence dextran. TNS-exposed monocytes were sliced and analyzed by scanning electron microscopy and energy dispersion type X-ray spectroscopy. The inner surface of vacuoles involved some agglomerated body, which showed the presence of titanium. These results indicate a unique toxicity of TNS, in which TNS entered into endocytic pathway leading to the formation of vacuoles, followed by caspase-dependent apoptosis.

2785 Toxicological Evaluation of Cobalt Oxide Nanoparticles on HepG2

G. Ozhan, M. Abudayyak and T. Altincekic Gurkaynak. *Istanbul University, Istanbul, Turkey.*

Cobalt oxide (Co3O4) nanoparticles in nanomedicine and nanotechnology especially is due to their enhanced magnetic properties, even though Co has been reported to have high toxicity and could potentially be classified as a carcinogen by the International Agency for Research on Cancer (IARC). Indeed, Co based nanoparticles reportedly produce inflammation, apoptosis, and DNA damage. Therefore, we investigated the cytotoxicity, genotoxicity, oxidative damage, and apoptosis-induction of Co3O4 nanoparticles (40 nm) on the liver (HepG2 hepatocarcinoma cell). After 24 h exposure, the nanoparticles decreased cell viability at ≤100 µg/mL, while increasing viability at the concentrations more than 100 µg/mL. The nanoparticles induced DNA (≤1.5-fold of the negative control) and oxidative damage with increased malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) levels and decreased glutathione (GSH) levels. The nanoparticles also slightly induced apoptosis/necrosis (≤ 6.3 fold of the negative control). Their adverse effects should raise concern about their safety associated with their applications in consumer products. However, the obtained results need to be supported with *in vivo* studies to fully understand the mechanism of Co3O4 nanoparticle toxicity.

2786 Surface Reactivity and Cell-Particle Bio-Interactions Explain the Precocious Cytotoxicity of Copper Oxide Nanoparticles

E. Moschini and P. Mantecca. *Department of Earth and Environmental Sciences, University of Milano Bicocca, Milan, Italy.* Sponsor: K. Savolainen.

Copper oxide nanoparticles (nCuO) are promising substitutes of different toxic substances currently used for antimicrobial purposes but is already proven that they are also strong cytotoxic agents for mammalian cells. The aim of this work was to understand how differently synthetised nCuO could induce precocious cytotoxic effects depending on their chemical-physical properties. In particular, we focused the attention on cell-particle interaction and protein modulation in order to define potential markers of precocious effects by exposing A549 cells to a commercial form of nCuO in comparison to sonochemically prepared-one. Particles were fully characterised by HRTEM, XRD, TCA and

ICP-MS. Cytotoxicity studies and oxidative stress biomarkers analyses were coupled with microscopic techniques (TEM, SEM and confocal microscopy) to describe the interactions at the bio-interface. In our conditions, we found that sonochemically synthetised nCuO induced stronger oxidative damages (protein carbonylation and thiol reduction) than commercial ones at very short exposure time (1h) and in this case, the internalization by the endocytic process was mandatory to start the cytotoxic pathway. This was confirmed by the viability results obtained by co-incubating cells with inhibitors of energy dependent mechanisms of uptake. On the contrary, the observed early cytotoxicity of the commercial nCuO was quite independent from the particle internalization and lysosomal dissolution. Different results were obtained by using dispersants to increase the stability of the particles, testifying that the protein corona effect plays a critical role in the toxicity pathway. All together, these findings suggest that the oxidative potential of nCuO is strictly associated to the size and surface properties of nanoparticles and is almost independent from ion dissolution. Finally our particles were both able to induce a significant oxidative burst by two different mechanisms and for sonochemical nCuO the process occurred faster driving cells to a precocious apoptotic cell death. This work was supported by Fondazione Cariplo (OverNanotox 2013-0987)

PS 2787 Carbon Nanoparticles Modulate Viral Infectivity: A Role for Sialic Acid Receptors

S. Hentschel[2], H. Chen[2], J. Loeb[2], J. Lednicky[2] and T. Sabo-Attwood[2]. [1]*Environmental and Global Health, University of Florida, Gainesville, FL and* [2]*Molecular Genetics and Microbiology, University of Florida, Gainesville, FL.*

Nanoparticles have unique characteristics that provide value to a growing number of consumer industries. Single-walled carbon nanotubes (SWNTs) are cylindrical allotropes of carbon that have previously been shown to increase the infectivity of pandemic H1N1 influenza virus (pH1N1) in small airway epithelial cells (SAECs). Our group has identified several mechanisms driving SWNT-induced viral infections including modulation of sialic acid (SA) receptors. SA participates in influenza entry into target cells. We designed experiments to investigate two questions: Do SWNTs alter the expression, localization, and function of SA receptors?; Do different types of carbon nanotubes modulate the receptors and viral infectivity similarly? To answer these questions, we characterized the expression of two SA linkages α2,3 and α2,6 on SAECs that are relevant to pH1N1 infections. Using immunohistochemistry, qPCR, and flow cytometry, we determined that the α2,3 linkage is more prevalent compared to the α2,6 linkage. Furthermore, expression levels of the sialyltransferase ST3GAL4, responsible for adding the α2,3 linkage, was greater than ST6GAL1 which adds α2,6 linkages. The α2,3 linkage is associated with lower respiratory tract epithelium and increases the virulence of influenza by transmission and secondary infections. These data provide evidence that SAECs are a good model system for studying changes in SA receptors by nanotubes and pH1N1. To determine the impact of various nanotubes on viral infectivity, SA receptors, and siayltransferase expression, SAECs were exposed to SWNTs or multi-walled nanotubes (MWNTs) and subsequently infected with pH1N1. Infectivity was measured by a TCID50 titer assay. The results indicate MWNTs caused a dose dependent decrease in viral infectivity up to ten fold where SWNTs demonstrated increased infectivity five fold. SWNTs also caused an observed increase in the expression and altered the localization of α2,6 SA receptor. We are currently determining whether similar patterns result during MWNT exposures. Overall, all carbon nanotubes do not have the same modulatory effect on viral entry and infectivity, which may be a result of changes in the α2,3 linked SA receptor in SAECs. These studies further highlight a novel mechanism of nanoparticle toxicity unidentified to date and should be further investigated.

PS 2788 Optimization of an Air-Liquid Interface Exposure System for Assessing Toxicity of Airborne Nanoparticles

S. I. Latvala[5], J. Hedberg[3], L. Möller[1], I. Odnevall Wallinder[3], H. L. Karlsson[2] and K. Elihn[5]. [1]*Karolinska Institutet, Huddinge, Sweden;* [2]*Karolinska Institutet, Stockholm, Sweden;* [3]*KTH Royal Institute of Technology, Stockholm, Sweden;* [4]*Stockholm University, Stockholm, Sweden and* [5]*Department of Environmental Science and Analytical Chemistry, Stockholm University, Stockholm, Sweden.* Sponsor: I. Ali.

The use of refined methods is currently needed for characterizing the toxicity of airborne nanoparticles. Due to the importance of respiratory tract as an exposure route for airborne nanoparticles in humans, we have developed an air-liquid interface (ALI) exposure system that can mimic pulmonary exposure *in vitro*. The system utilizes electrostatic force in order to enhance the deposition of nano-sized (1-100 nm) metal particles on lung cell cultures. Compared to traditional submerged systems, the use of airborne particles allows more realistic exposure conditions for characterizing toxicological effects induced by airborne nanoparticles. The purpose of this study was to investigate how the deposition of silver nanoparticles (AgNPs) is affected by different settings of the ALI system. Additionally, the viability and metabolic activity of A549 cells was studied following AgNP exposure. Particle deposition increased markedly with increasing aerosol flow rate and electrostatic field strength. The highest amount of deposited particles (2.2 µg/cm2) at cell-free conditions following 2 h exposure was observed for the highest flow rate (390 mL/min) and the strongest electrostatic field (±2 kV). This was estimated corresponding to deposition efficiency of 94 %. Cell viability (AlamarBlue-assay) was not affected after 2 h exposure to clean air in the ALI system. Cells exposed to AgNPs (0.45 and 0.74 µg/cm2) for 2 h showed significantly ($p<0.05$) reduced metabolic activities (64 and 46 %, respectively). Our study shows that the ALI exposure system can be used for generating more realistic conditions for *in vitro* exposures, which enables improved mechanistic and toxicological studies of nanoparticles in contact with human lung cells.

PS 2789a Cerium Oxide Nanoparticles Suppress Differentiation of Murine Neuronal Stem Cells

A. R. Gliga[4], K. Edoff[4], F. Caputo[2], T. Källman[3], H. L. Karlsson[4], L. Ghibelli[2], E. Traversa[2], S. Ceccatelli[4] and B. Fadeel[4]. [1]*Department of Biology, University of Rome Tor Vergata, Rome, Italy;* [2]*Department of Chemical Science and Technology, University of Rome Tor Vergata, Rome, Italy;* [3]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden and* [4]*Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden;* [5]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

Cerium oxide nanoparticles (nanoceria) have shown promise as antioxidants both *in vitro* and *in vivo*. The aim of the present study was to investigate the effects of nanoceria on neural progenitor cells, using the murine cell line C17.2. First, we assessed the effects of nanoceria vs. samarium (Sm) doped nanoceria (displaying a reduced antioxidant activity) on C17.2 cell viability in the presence of an oxidative insult (DMNQ, dimethoxy-naphthoquinone). Cellular uptake was quantified by ICP-MS, intracellular localization was confirmed by TEM and cell viability was monitored by automated microscopic morphological assessment (Cell-IQ assay). Both nanoparticles were taken up to a similar extent by proliferating and differentiating C17.2 cells and were shown to be non-cytotoxic. Nanoceria, but not Sm-doped nanoceria elicited a cytoprotective effect after 8 h, but not after 12 h of DMNQ exposure. Next, we assessed the effects on neuronal differentiation, which was induced by serum deprivation and N2 growth factor supplementation. Neuronal differentiation of the C17.2 cell line was quantified by immunofluorescence (β3-tubulin) and next-generation sequencing (RNA-Seq) was employed to determine genome-wide changes at transcriptional level. Nanoceria, but not the Sm-doped particles, reduced β3-tubulin expression, an early marker for neuronal differentiation. Additionally, network analysis of the RNA-Seq data using the Ingenuity Pathway Analysis tool revealed that nanoceria significantly reduced expression of genes involved in neuronal development and cell differentiation. Taken together, the present *in vitro* study has shown that nanoceria provides temporary cytoprotection against oxidative insult, but has also revealed potential developmental neurotoxicity, as evidenced by the inhibition of neural differentiation of C17.2 cells.

PS 2789 *In Vitro* Percutaneous Penetration of Branched Polyethyleneimine (bPEI) and Polyethylene Glycol (PEG) Coated Silver Nanoparticles (AgNPs) into Human Skin: A Mass Balance Study

V. D. Topping, Z. M. Keltner, R. L. Sprando, J. J. Yourick and M. E. Kraeling. *Office of Applied Research and Safety Assessment, US FDA, Laurel, MD.*

In previous studies, we found that positively charged (bPEI-coated) and neutral (PEG-coated) 20nm AgNPs penetrated into human skin more than negatively charged (citrate-coated) AgNPs. To determine the extent of silver penetration throughout the layers of the skin, we conducted an *in vitro* mass-balance dermal penetration study using AgPEG and AgbPEI. Frozen human abdominal skin was obtained from three cadavers. The skin was dermatomed, punched into skin disks and placed in flow-through diffusion cells. Barrier integrity of skin was confirmed prior to dosing. AgNPs were characterized to confirm size, shape, and agglomeration state, and dosed on the skin in a 0.01% aqueous solution containing AgbPEI or AgPEG. After 24 hours, the skin surface was

washed with a 1% soap solution and unabsorbed AgNPs were removed with cotton swabs. Skin disks were tape-stripped twice to remove surface-bound AgNPs and additional tape-strips (~11) removed the remaining stratum corneum. Epidermis was heat separated from dermis. Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure total silver content (AgNPs and free silver ions) of cotton swabs, tape-strips, skin fractions, receptor fluid, and parafilm on which skin was handled. The results (percent of applied dose) showed that the majority of AgNPs were washed from the skin surface. The silver that penetrated skin remained mostly in the stratum corneum (3.5% for AgbPEI and 1.7% for AgPEG) with lesser amounts absorbing into the epidermis (1.2% AgbPEI and 0.4% AgPEG) and dermis (0.06% AgbPEI and 0.04% AgPEG). No silver was detected in the receptor fluid. Average recovery of the dosed AgNPs was 84.4 to 89.9% for AgPEG and AgbPEI, respectively. Results from this study suggest there is minimal dermal absorption of AgNPs. This data will be useful for evaluating the safety of cosmetic products containing AgNPs.

2790 Abstract Withdrawn by Author.

2791 Brain Inflammation and Blood Brain Barrier Disruption after Sub-Acute Inhalation Exposure to Titanium Dioxide Nanoparticle Aerosol in Aging Rats

C. Disdier[1], M. Chalansonnet[2], F. Gagnaire[2], L. Gate[2], F. Cosnier[2], J. Devoy[2], W. Saba[2], E. Brun[3], A. Lund[4] and A. Mabondzo[1]. *[1]CEA, Gif sur Yvette, France; [2]INRS, Nancy, France; [3]Laboratoire de Chimie Physique, UMR 1023, Orsay, France and [4]University of North Texas, Department of Biological Sciences, Texas, TX.*

Notwithstanding potential neurotoxicity of Titanium dioxide nanoparticles (TiO2 NPs), the toxicokinetics and consequences on blood brain barrier (BBB) functions remain poorly characterized. We previously described the biodistribution of intravenous administered TiO2 NPs and highlighted a modulation of BBB physiology associated with neuro-vascular inflammation. To improve risk assessment we needed to evaluate impact on BBB under realistic environmental conditions and to take into account vulnerability status such as age. Adult and aging rats inhaled a 10mg/m3 concentration of TiO2 NPs aerosol for 28 days, 5 days a week, 6 hours per day. Blood and tissues were collected at various times and Ti levels were measured. *In vivo* BBB permeability was evaluated as well as expression of structural BBB components on isolated brain micro-vessels. The brain inflammatory profile was assessed by multiplex analysis, RT-PCR and localized on brain slides by immuno-fluorescence. After a 4-week inhalation period, TiO2 NPs were observed to accumulate in the lungs, with a gradual clearance over the time points measured. No age-related differences in biodistribution were noted in the current studies. Moreover, we reported an age dependent increase in BBB permeability which underscores the brain vulnerability of aging population. We also noted an age-dependent modulation of BBB integrity parameters suggesting a BBB breakdown in vulnerable aging rats. Modulation in expressions of ABC and SLC transporters at the BBB level were highlighted suggesting alteration of detoxification function of the BBB. Ti biopersistence in lung tissues was associated with significant increase of cytokines/chemokines in the brain such as interleukine 1β, interferon γ or fractalkine. These brain inflammation markers suggest indirect induction of brain inflammation that seems dependent on circulating mediators. Despite lack of CNS translocation, we suggested a link between the presence of TiO2 NPs in organs and dysregulation of BBB physiology.

2792 TMAT-AuNP Core Size is Inversely Correlated to Toxicity in the Zebrafish Embryo

M. A. Garland[2], L. Truong[2], T. Zaikova[1], J. E. Hutchison[1], K. Kim[3] and R. L. Tanguay[2]. *[1]Department of Chemistry and the Materials Science Institute, University of Oregon, Eugene, OR; [2]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR and [3]Department of Environmental Engineering, Seoul National University of Science and Technology, Seoul, Korea, Republic of.*

Gold nanoparticles (AuNPs) are increasingly used in a variety of commercial and industrial products. There are opportunities to optimize performance and safety using precision engineering since various physical attributes of AuNPs such as core size and surface functionality can be controlled. We previously evaluated the bioactivity of a library of AuNPs and identified differential biocompatibility from a series of particles. To systematically evaluate the biocompatibility of AuNPs with small core diameter changes, we synthesized a series of AuNPs with core sizes ranging from 0.8 to 5.8 nm, each fully functionalized with N,N,N-trimethylammoniumethanethiol (TMAT). We exposed zebrafish (*Danio rerio*) embryos at 6 hours post fertilization (hpf) to quarter-log concentration increments of each AuNP. We evaluated mortality and morphology at 24 and 120 hpf using 22 total endpoints, and assessed behavioral response to stimulus at 120 hpf. We found that small- and intermediate-sized TMAT-AuNPs induced the highest mortality and morphological defects in a threshold-dependent manner. Aberrant behavioral responses were only detected in fish exposed to the smallest TMAT-AuNP. From these data we conclude that, as a general trend, the toxicity of TMAT-AuNPs is inversely correlated to their size. Using these results, we can begin to elucidate the mechanisms of toxicity related to the physical attributes of AuNPs. Supported by NSF grant #1512755 and NIH P30ES000210.

2793 Effects of Prenatal Inhalation of Copper Nanoparticles on Murine Dams and Offspring

A. Adamcakova-Dodd, M. M. Monick, L. S. Powers, K. N. Gibson-Corley and P. S. Thorne. *Internal Medicine, University of Iowa, Iowa City, IA; Occupational and Environmental Health, University of Iowa, Iowa City, IA; Pathology, University of Iowa, Iowa City, IA.*

Increasing numbers of individuals may be exposed to nanomaterials during pregnancy. We sought to determine if prenatal inhalation exposure to copper nanoparticles (Cu NPs) has an adverse effect on dams and offspring, including an analysis of inflammatory markers (Th1/Th2 cytokine profiles). Physicochemical characterization of Cu NPs was performed. Dams and non-pregnant mice (C57Bl/6 J) were exposed to Cu NPs or laboratory air in whole-body exposure chambers for 4 hrs/day on gestation days (GD) 3-19 (3.5 mg/m3). Mice were euthanized on GD 19 or 7 weeks later. Bronchoalveolar lavage (BAL) fluid was analyzed for total and differential cells. Cytokine/chemokine concentrations were determined in the BAL fluid and the plasma of dams/non-pregnant mice and pups. Cu content was determined in selected tissues. Lungs and placentas were evaluated for histopathological changes. Gene expression of the Th1/Th2 profiles were analyzed in spleens of pups. The survival rate of 7 week old pups exposed to Cu NPs was significantly lower than control pups (73 vs. 97 %). Both dams and non-pregnant mice exposed to Cu NPs had significant pulmonary inflammation with increased number of neutrophils in the BAL fluid compared to controls. Perivascular lymphoplasmacytic cuffing was found in the lungs of exposed mice and was more pronounced in the non-pregnant group. Levels of inflammatory cytokines/chemokines fluid were significantly higher in non-pregnant than pregnant exposed mice. Histopathology evaluation of placentas did not identify any pathological changes. No translocation of Cu into the placenta or the fetus was found. Expression of several Th1/Th2 or other immune response genes in pups' spleens were found to be significantly up- or down-regulated. Prenatal exposure to Cu NPs caused a profound pulmonary inflammation in dams and strong immunomodulatory effects in offspring. There was no clear polarization of genes expressed in pups' spleens towards Th1 or Th2 type of response. Supported by NIEHS/NIH P30 ES005605

2794 Biochemical and Histopathological Evaluation of PEG-Coated and Uncoated Gold Nanoparticles in Sprague-Dawley Rats

A. Patlolla and P. Tchounwou. *Jackson State University, Jackson, MS.*

Nanoparticles (NPs) offer a great possibility for biomedical application, not only to deliver pharmaceutics, but also to be used as novel diagnostic and therapeutic approaches. Currently, there are no data available regarding to what extent the degree of the toxicity and the accumulation of gold nanoparticles (GNPs) are present in *in vivo* administration. The aim of this study was to assess the effects, after oral administration, of poly-ethylene-glycol (PEG) coated and non-coated GNPs on various hepatotoxicity markers such as alanine (ALT), aspartate aminotransferases, alkaline phosphatase, induction of reactive oxygen species and histopathological analysis in the mouse model. Sprague -Dawley rats were exposed to four different concentrations of PEG-coated and non-coated GNPs (12.5, 25, 50 and 100 µg/kg BW) and a control. Samples were collected 24 hours after the last treatment following standard protocols. Exposure to PEG-coated and non-coated GNPs enhanced the activities of serum amino-transferases (ALT/AST), alkaline phosphatases (ALP) and concentration of lipid hydro peroxide compared to control. Histopathology of exposed liver showed a statistically significant effect in the morphological alterations of the tissue compared to controls. However, PEG-coated GNPs demonstrated enhanced hepatotoxic effect than non-coated GNPs. The cellular findings reported here do suggest that both GNPs has the potential to induce hepatotoxicity in Sprague-

Dawley rats through activation of the mechanisms of oxidative stress, which is of sufficient significance to warrant *in vivo* animal exposure studies. However, more studies to clarify the role of encapsulation in the *in vivo* toxicity of GNPs, are required and parallel comparison is preferred.

2795 Toxic Effects of Oral Exposure to Silver Nanoparticles in Rats

T. Garcia, D. Lafuente, J. Blanco, M. Gómez, D. J. Sánchez and J. L. Domingo. *Toxicology, Universitat Rovira i Virgili, Reus, Spain.*

Because of its small size (1–100 nm), silver nanoparticles (AgNPs) have unique physical and chemical properties, which make them desirable materials in biological studies. However, these properties are also responsible for specific toxicity. The aim of this study was to determine whether an *in vivo* oral sub-chronic exposure to PVP-AgNPs, could be harmful for the general health status of Sprague Dawley rats. Animals were orally given 0, 50, 100 and 200 mg/kg/day of PVP-AgNPs. Body and organ weight, silver accumulation and excretion, biochemical and hematological parameters, as well as histopathological changes and subcellular distribution were examined. In addition, metal (Fe, Zn, Cu, Mg) accumulation and distribution in different tissues were also determined. Results show that after 90 days of treatment with PVP-AgNPs, the highest concentration of silver was found in the ileum. Silver was mainly excreted through the feces, being found in urine only few amounts of this element. PVP-AgNPs did not induce dose-related changes on different biochemical and hematological parameters. AgNPs were found in hepatic and ileum cells. Silver accumulation in liver of treated animals resulted in a higher number of binucleated hepatocytes and increased proliferating cells. On the other hand, significant differences in Cu and Zn levels were found in liver, thymus and brain of PVP-AgNPs-treated rats. The current results indicate that oral sub-chronic treatment of PVP-AgNPs may induce harmful effects in orally exposed rats.

2796 Toxicity Assessment of Model Chemical Mechanical Planarization Slurries Using *Daphnia magna*

S. Karimi[2], R.-H. Wang[2], P. Pantano[2] and R. Draper[2]. [1]*Chemistry and Biochemistry, The University of Texas at Dallas, Richardson, TX and* [2]*Chemistry and Biological Sciences, The University of Texas at Dallas, Richardson, TX.*

The goal of this study was to determine the acute toxicity to *Daphnia magna* (*D. magna*) of four model nanoparticle (NP) slurries: colloidal silica (c-SiO2), fumed silica (f-SiO2), ceria (CeO2) and alumina (Al2O3). The semiconductor manufacturing industry uses these NPs as abrasives in chemical mechanical planarization (CMP) processes to polish wafer surfaces to high tolerances. Since the CMP NP slurries are used by the semiconductor industry in large quantities, it is of interest to study the impact of the NP slurries on aquatic organisms. Assessing the toxicity of real-world CMP slurries is problematic due to the toxic constituents they are known to contain, such as oxidants, surfactants, and biocides. The model slurries used in this study were provided by a CMP supplier (CABOT microelectronics) and were specially formulated to eliminate toxic additives that might confound toxicity studies, thus focusing on the potential toxicity of the metal oxide NPs themselves. *D. magna* is a fresh water flea known to be sensitive to ecological pollutants and is a commonly used model organism for ecotoxicity studies. In this study, static acute toxicity tests were conducted in accordance with standard EPA methods. *D. magna* neonates (less than 24h old) were exposed to model NP slurries at various concentrations for 96h. At the end of the incubation, morbidity was recorded and the sizes of the surviving daphnia were measured. Preliminary results showed that f-SiO2 was not toxic to *D. magna* and no significant difference in body sizes were observed at concentrations up to 5 mg/mL. Similarly, c-SiO2 was not toxic up to 4 mg/mL but the body sizes of c-SiO2 treated *D. magna* were slightly larger than the untreated controls. Unlike c-SiO2, CeO2 showed no morbidity up to 2 mg/mL; however, *D. magna* body sizes decreased as a function of CeO2 concentration. Al2O3 was the only NP among the four that showed acute lethal toxicity to *D. magna* with a 96h LC50 of 1.1 ± 0.2 mg/mL. Moreover the body sizes of *D. magna* exposed to Al2O3 decreased nearly 53% when exposed to the Al2O3 slurry at 1.5 mg/mL. Overall, the results of this study demonstrated that different model CMP NPs exert different acute toxic effects on *D. magna* morbidity or growth, or both.

2797 Mixture Effects of Titanium Dioxide Nanoparticles and Bisphenol A on Toxicity to Developing Zebrafish

L. Denluck[2], Z. Klocke[2] and S. Harper[2]. [1]*Chemical, Biological, and Environmental Engineering, Oregon State University, Corvallis, OR and* [2]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

The ability of titanium dioxide nanomaterials to transform organic compounds presents a powerful tool for groundwater remediation and water treatment purposes. Yet, it is important to understand the interactions occurring between nanomaterials and contaminants before widespread implementation in order to prevent unforeseen consequences. These can include altered toxicity of the particle or the contaminant as a result of their interaction or transformation. In this work, coexposures of bisphenol A (BPA) with four titanium dioxide nanomaterials (anatase, rutile, 70% anatase/30% rutile, and manganese-doped) were employed to evaluate toxicity caused by individual contaminants or mixtures of co-contaminants. Embryonic zebrafish were dechorionated at 8 hours post-fertilization (hpf) and exposed to 2, 6, 10, 16, and 20 mg/L of BPA with 10 mg/L of each nanomaterial. Mortality was assessed every 24 hours for 5 days, and sublethal toxicity was assessed at 24 hpf and 120 hpf. Coexposures resulted in differential toxicity when compared to individual exposures, with anatase and manganese-doped particles causing a significant decrease in toxicity in comparison to BPA alone. To investigate the effect of BPA on the behavior of the different nanomaterials, the size of each nanoparticle in the presence of varying concentrations of BPA was monitored via dynamic light scattering over a 24 hour period. Addition of BPA modified the rate of size change over time, causing different rate effects for each particle type. We hypothesize that a concentration-dependent surface interaction between BPA and the nanomaterials caused the altered toxicity to the developing embryo. These findings demonstrate that the coexistence of titanium dioxide nanomaterials and BPA may reduce the toxicity of BPA to aquatic species, further validating the use of titanium dioxide nanomaterials for water remediation purposes; however, further study is needed to fully elucidate the mechanism behind the antagonistic effects.

2798 The Role of Inflammasome in Pulmonary Injury Caused by Inhalation Exposure to Tungsten (IV) Oxide (WO_3) Nanoparticles in Golden Syrian Hamsters

M. Prajapati and J. M. Cerreta. *Department of Pharmaceutical Sciences, St. John's University, Queens, NY.*

Tungsten is a metal with many applications. Tungsten (IV) oxide (WO_3) nanoparticles (NP) are generated in many industrial processes. Widespread use of tungsten has raised concerns about possible occupational and environmental toxicity. However, the toxicity of nano-tungsten has not been addressed. To investigate the potential deleterious effects of inhalation exposure to WO_3 NP, animals were divided into three groups - control, exposed to sterile distilled water and two treatment groups exposed to either 5 or 10 mg/m3 of aerosolized WO_3 NP for 4 hrs/day for 7 days in a whole body exposure chamber. WO_3 NP were characterized by TEM and the chamber concentration was monitored using the NanoScan SMPS. Animals were euthanized 24 hours post exposure. Lung tissues were either fixed in gluraldehyde or snap frozen in liquid nitrogen for biochemical analysis. For scanning electron microscopy, fixed tissues were processed by the critical point method. When compared to controls, scanning electron micrographs indicated the presence of membrane blebs in the irritated airways. Transmission electron microscopy also revealed the localization of WO_3 NP in alveolar epithelial cells. Western blot analysis indicated a 1.5 and 1.7 fold increase in NLRP3 (NOD-like receptor family, pyrin domain containing 3) in the treatment groups (5 and 10 mg/m^3 respectively) as compared to controls. Levels of cathepsin B were 1.3 fold greater in lung tissue exposed to 5 and 10 mg/m^3 WO_3 NP. Results from these experiments indicate that exposure to WO_3 NP can induce ultrastructural changes in lungs as seen in electron micrographs. The presence of NLRP3 and cathepsin B indicate the activation of inflammasome pathway. A possible molecular mechanism of injury is through activation of caspase-1 induced pyroptotic cell death as indicated by an increase in caspase-1 and IL-1β.

2799 Differential Effects of Silver Nanoparticles (AgNP) and Silver Acetate (AgOAc) on the Accumulation, Distribution, and Potential Toxicity of Silver in Rats Following Daily Oral Administration for 13-Weeks

M. D. Boudreau[4], M. S. Imam[4], A. M. Paredes[4], M. S. Bryant[4], R. P. Felton[4], C. K. Cunningham[4], M. Y. Jones[4], K. J. Davis[1] and G. R. Olson[1]. *[1]Toxicologic Pathology Associates, Jefferson Laboratories, Jefferson, AR; [2]Bioinfomatics and Biostatistics Division, National Center for Toxicological Research, US FDA, Jefferson, AR; [3]Division of Biochemical Toxicology, National Center for Toxicological Research, US FDA, Jefferson, AR and [4]NCTR-ORA Nanotechnology Core Facility, National Center for Toxicological Research, US FDA, Jefferson, AR.* Sponsor: M. Boudreau.

AgNP have broad applications in consumer products, and reports indicate that ingested AgNP distribute to numerous organs in mammals. This study examined particulate and ionic forms of silver and particle size for differences in tissue silver accumulation, distribution, morphology, and potential toxicity when administered by daily oral gavage to Sprague-Dawley rats for 13 weeks. Test materials and dose formulations were characterized by transmission electron microscopy, dynamic light scattering, and inductively-coupled mass spectral analyses. Seven-week-old rats (10 rats per sex per group) were randomly assigned to treatments: AgNP (10, 75, 110 nm) at 9, 18, and 36 mg/kg; AgOAc at 100, 200, 400 mg/kg; and controls (2 mM sodium citrate (CIT) or water). At termination, complete necropsies were conducted, histopathology along with hematology, serum chemistry, micronuclei and reproductive system analyses were performed, and silver accumulations and distributions in tissues were determined. Male and female rats exposed to AgNP did not show any significant changes in body weights or feed and water intakes relative to controls. Hematology and serum chemistry values, micronuclei, reproductive system analyses, and absolute or relative organ weights did not differ from controls. Distributional and morphological differences of silver were observed by transmission electron microscopy for 10 nm AgNP and AgOAc, and silver showed significant dose-dependent and AgNP size-dependent accumulations in all tissues examined. Furthermore, sex differences in silver accumulations were noted for a number of tissues and organs, with accumulations significantly higher in female rats, especially in the kidney, liver, jejunum, and colon.

2800 Effects of Pre-Exposure Dispersion Status on Nanoparticle Distribution and Fibrosis in the Lung

T. M. Sager[1,2], M. Wolfarth[1], D. W. Porter[1], R. Mercer[1], V. Castranova[3] and A. Holian[2]. *[1]NIOSH, Morgantown, WV; [2]CEHS, University of Montana, Missoula, MT and [3]School of Pharmacy, West Virginia University, Morgantown, WV.*

An important aspect of nanoparticle toxicity is the deposition and translocation of the particles once they reach the lung. In the form of agglomerates deposition characteristics can change, as the agglomerates will have a greater aerodynamic diameter than singlet nanoparticles. We hypothesize that pre-exposure dispersion status of the nanoparticle will correlate with particle deposition and translocation within the lung. Specifically, we postulate that well dispersed (WD) particles will be more likely to move into the interstitial space of the lung, while poorly dispersed (PD) particles will primarily be taken up by the alveolar macrophages (AMs). To test our hypothesis, nano-sized NiO was suspended in four different dispersion media (PBS, dispersion medium (DM), Survanta, or Pluronics F-68). At each respective dose, WD and PD suspensions (sonicated at 25W continuous output, 20 min or 5 min, respectively) were created. Mice (male, C57BL/6J, 7 weeks old) were given 40 or 80 µg/mouse of nano-sized NiO in the different states of dispersion via pharyngeal aspiration. At 2 hours, 7, and 56 days post-exposure, lungs were collected and fixed by intratracheal perfusion. Lung sections were then viewed using a CytoViva microscope. At 2 hours post-exposure both PD and WD NiO structures were primarily distributed in the airspaces of the first few alveolar generations. However, the forms of distribution were quite different between PD and WD NiO at 7 and 56 day post-exposure. The majority of the PD particles were in large clumps in the airspaces. Many of the large clumps induced granulomatous nodules. The majority of WD NiO particles were in AMs or found within the interstitium. By 7 and continuing out to 56 days post-exposure, there was significant fibrotic development within the granulomatous nodules with PD NiO. However, granulomatous nodules and the associated fibrotic development were not observed with WD NiO particles. This work was supported by NIH grant F32 ES021341.

2801 Inhaled TiO_2 Nanoparticles Induce Morphologic and Biochemical Changes in the Lungs of Golden Syrian Hamsters

O. O. Adebolu and J. M. Cerreta. *Department of Pharmaceutical Sciences, St. John's University, Queens, NY.*

TiO_2 is a metal oxide that has found diverse applications from sunscreens to food additives. Inhaled metal nanoparticles have been shown to deposit in the parenchyma of the lungs causing generation of ROS, inflammation, cell death and fibrosis. NIOSH has classified TiO_2 as a potential carcinogen following development of tumors after chronic inhalation in rats exposed to 250mg/m^3 of TiO_2. From such studies NIOSH set an exposure limit of 0.3mg/m^3 while OSHA set a permissible exposure limit of 15mg/m^3. The current study investigated the toxicity of TiO_2 NP in Golden Syrian Hamsters. Hamsters were exposed in a whole body chamber to aerosolized vehicle (control) or aerosolized TiO2 NP at concentrations of 0.3mg/m^3, 3mg/m^3 or 15mg/m^3 (4 hrs/day for 14 days). 24hrs post exposure, hamsters were euthanized, lungs were lavaged and either fixed or frozen for further analysis. Bronchoalveolar lavage was assessed for levels of LDH, SOD and TNF-α. Average size of TiO_2 NP was determined to be 36.9 ±14.4nm by TEM evaluation. Lavage from the lungs of hamsters treated with 0.3, 3.0 or 15mg/m3 NP had a 33%, 73% or 138% increase in TNF-α as compared to controls. SOD levels were significantly higher by 137% (3mg/m^3 group) and 337 % (15mg/m^3 group) while LDH levels were increased by 73% and 108% (3mg/m3 and 15mg/m3 groups respectively) as compared to controls. H & E evaluation of lung tissue revealed significant hyperplasia in animals exposed at 3mg/m3 and 15mg/m^3 while TUNEL assay revealed the presence of apoptotic cells in the 15mg/m^3 group. Scanning electron micrographs of animals exposed to TiO_2 NP showed presence of blebbing in airways while the control group had cells that appeared normal. Such findings indicate that inhalation of nanosized TiO_2 particles induce apoptosis and histopathological changes in lung tissue, increase in SOD levels suggest induction of oxidative stress while the increases in LDH and TNF-α indicate induction of parenchymal injury.

2802 Effect of Ascorbic Acid Pretreatment on Brain Perivascular Injury Induced by Prenatal Exposure to Carbon Black Nanoparticle in Mice

A. Onoda[1,3], K. Takeda[3] and M. Umezawa[3]. *[1]JSPS Research Fellow, Tokyo, Japan; [2]Cent. Env. Health Next Gen., RIST, Tokyo University of Science, Chiba, Japan and [3]Grad. School Pharm. Sci., Tokyo University of Science, Chiba, Japan.* Sponsor: K. Nohara.

Objective: Our previous report* showed that maternal exposure to carbon black nanoparticle (CB-NP) alters histological feature of the brain perivascular macrophages and astrocytes of mouse offspring. The aim of the present study was to investigate protective effects of ascorbic acid, one of the antioxidant reagents, on the brain perivascular cell damages induced by maternal exposure to CB-NP in offspring mice. Methods: Pregnant ICR mice were treated with CB-NP suspension without bulk agglomerates (95, 73, 15, 2.9 µg/kg/time) by intranasal instillation on gestational days 5 and 9. Ascorbic acid (500 mg/kg) were intraperitoneally administered at 1 hour before every administration of CB-NP. Brains and dissected frontal cortex were collected from offspring mice at 6 and 12 after birth. Phenotypes of perivascular cells were examined by western blotting for glial fibrillary acidic protein (Gfap) and aquaporin-4 (Aqp4), immunohistochemistry, comprehensive gene expression profiling by microarray, and gene ontology (GO)-based annotation analysis. Results: Quantitative analysis by western blotting indicated that the expression levels of Gfap and Aqp4 were dose-dependently increased in the frontal cortex by CB-NP. Gfap expression was increased in the astrocytic endfoot attached to denatured perivascular regions in the gray matter and Aqp4 expression was increased in brain parenchyma, not in perivascular region, of frontal cortex. The increase in Gfap and Aqp4 protein expressions levels were not suppressed by ascorbic acid pretreatment. Analysis of gene expression using GO terms indicated that expression levels of genes associated with angiogenesis, chemotaxis, and cell proliferation were altered in the frontal cortex of offspring by CB-NP maternal exposure, and these gene expression levels were not altered by ascorbic acid pretreatment. Conclusion: The present study indicated that maternal exposure to CB-NP induced chronic perivascular injury with an increase in Gfap and Aqp4 of frontal cortex, which was not protected by ascorbic acid pretreatment. The present study suggests that the mechanism of brain perivascular injury induced by prenatal exposure CB-NP may not related to increase in oxidative stress of a pregnant mother. *Onoda A et al., PLoS One. 2014.

2803 Exposure to Respirable Biodiesel/Diesel Blend (BD50) Exhaust Particulate Generates Pronounced Aberrations in Male Reproductive System

E. R. Kisin[1], N. Yanamala[1], M. T. Farcas[1], D. W. Gutkin[4], M. R. Shurin[4], V. E. Kagan[2], A. D. Bugarski[3] and A. A. Shvedova[1,5]. *[1]EAB, NIOSH, Morgantown, WV; [2]Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA; [3]Office of Mine Safety and Health Research, NIOSH, Pittsburgh, PA; [4]Pathology, UPMC, Pittsburgh, PA and [5]Physiology and Pharmacology, WVU, Morgantown, WV.*

Biodiesel (BD) is considered an environmentally friendly, renewable fuel produced from vegetable and seed oils, animal fats and/or waste oils. Blends of BD with conventional hydrocarbon-based petroleum diesel (PD) are most commonly distributed for use in the fuel marketplace. Exposure to particles found in diesel exhaust (DE) has been reported to cause respiratory disease, cardiovascular disease and lung cancer. The disruption of male reproductive function has also been observed after exposure to DE particulates. To address whether respirable BD influences the murine male reproductive system, we compared the adverse effects of BD50 and PD exhaust particulate in C57BL6 mice. We found that pulmonary exposure to BD50 particulate significantly altered sperm integrity including concentration, motility and morphological abnormalities during the time course of post exposure. Moreover, BD50 caused up-regulation of inflammatory cytokines, increase in testicular testosterone, and reduction of serum luteinizing hormone. Testicular histopathology revealed interstitial edema, clustering of the dystrophic seminiferous tubules with arrested spermatogenesis and the presence of degenerating spermatocytes. Additionally, we observed that exposure to inhalable BD50 particulate caused severe oxidative stress and DNA damage in male reproductive organs as compared to PD. Overall, these results demonstrate that exposure to respirable BD50 exhaust particulate, in comparison to PD, generates pronounced adverse effects on male reproductive function.

2804 Genotoxic Effects of Cobalt Nanoparticles *In Vivo*

Y. Mo[1], R. Wan[2], Y. Zhang[1], A. Gu[3], D. Tollerud[1] and Q. Zhang[1]. *[1]Department of Environmental and Occupational Health Sciences, University of Louisville, Louisville, KY; [2]Department of Pathology, Fujian Medical University, Fuzhou, China and [3]Institute of Toxicology, Nanjing Medical University, Nanjing, China.*

Our and other groups have demonstrated that exposure to cobalt nanoparticles (Nano-Co) caused oxidative stress and inflammation *in vitro* and *in vivo*, which have been shown to be strongly associated with genotoxic and carcinogenic effects. However, few studies have been reported on Nano-Co-induced genotoxic effects *in vivo*. Here, we propose that Nano-Co may have genotoxic effects due to its high capacity for causing oxidative stress and inflammation. Our results showed that exposure of mice to Nano-Co (50 µg/mouse) for one week resulted in extensive lung inflammation. Ki-67 and proliferating cell nuclear antigen (PCNA) were stained by immunohistochemical methods to determine cell proliferation. Our results showed that increased Ki-67 and PCNA staining was observed in bronchiolar epithelial cells and hyperplastic type II pneumocytes in mouse lungs exposed to Nano-Co for one week. After four months of treatment, extensive interstitial fibrosis and proliferation of interstitial cells with inflammatory cells infiltrating the alveolar septa were observed. In the thickened alveolar walls, bronchiolization of the alveoli was observed, which was characterized by replacement of alveolar epithelial cells with bronchiolar-type epithelium and sometimes micro-papillomatosis. A large number of macrophages lined the alveolar walls, and emphysematous changes appeared. Increased nuclear Ki-67 and PCNA positive immunostaining was also found in some bronchiolar epithelial cells and bronchiolized cells in lungs from mice treated with Nano-Co. Moreover, exposure of mice to Nano-Co for three and six months caused increased level of 8-OHdG in genomic DNA of mouse lung tissues. Our results suggest that exposure to Nano-Co resulted in oxidative stress and lung inflammation and cell proliferation. In addition, gpt delta transgenic mice were intratracheally instilled with Nano-Co for four months and the mutant frequency and mutation spectrum in gpt gene was determined. Our results showed that Nano-Co induced a much higher mutant frequency as compared to controls, and the most common mutation was G:C to T:A transversion, which may be explained by Nano-Co-induced formation of 8-OHdG. These findings have important implications for understanding the potential health effects of nanoparticle exposure.

2805 Effects of Different Sizes of Silver Nanoparticles on *Gammarus fossarum* (Crustacea, Amphipoda): Link Between Physiological and Behavioural Responses

A. C. Gutleb[3], K. Mehennaoui[3], A. Georgantzopoulou[2,3], S. Cambier[3], G. Maël[4], J. Andreï[4], V. Felten[4], T. Serchi[3], S. Contal[3], J. Ziebel[3], C. Guignard[3], Y. Balachandran[1], S. Pain-Devin[4] and L. Giambérini[4]. *[1]Bharathiar University, Coimbatore, India; [2]NIVA, Oslo, Norway; [3]ERIN, Luxembourg Institute of Science and Technoogy, Esch-sur-Alzette, Luxembourg and [4]LIEC, University Lorraine, Metz, France.*

Despite the growing number of studies on the toxicity of AgNPs, their mechanisms of action in aquatic organisms are still poorly understood. The aim of this study was to investigate the toxic effects of different sizes of "conventionally synthesized" (AgNPs 20 and 200 nm) and "green synthesized" (making use of plant extracts avoiding solvents and other hazardous chemicals) (AgNPs 23 and 27 nm) particles on survival, physiological and behaviour responses of *Gammarus fossarum*. A first experiment aimed at determining the toxicity of AgNPs and Ag+, and to compare the sensitivity of two different populations of *Gammarus fossarum*. The first set of animals (Gf1) was collected at La Maix (Eastern France) and the second one (Gf2) was collected at the Schwaarzbaach (Colmar-Berg, Luxembourg). After sexing, males were kept and following a ten-day acclimatization period, animals were exposed during 72h to Ag+, AgNPs 23nm (1 - 8 µg.L-1), AgNPs 27 nm (1 - 100 µg.L-1), AgNPs 20 and AgNPs 200 nm (10 -1000 µg.L-1). The results showed that Ag+ (LC50Gf 1 = 3.9 µg.L-1, LC50Gf 2 = 2,3 µg.L-1) was the most toxic form of silver for both populations, followed by AgNPs 23 nm (LC50Gf 1 = 7.7 µg.L-1, LC50Gf 2 = 4.9 µg.L-1), AgNPs 27 nm (LC50Gf 1 > 100 µg.L-1, LC50Gf 2 = 5.5 µg.L-1) and AgNPs 20 nm (LC50Gf 1 = 835 µg.L-1, LC50Gf 2 > 1000 µg.L-1). Gf2 appeared to be the most sensitive population and was selected for the second experiment. Exposure to the highest concentration of Ag+ and AgNPs 23 nm (3 µg.L-1) led to a significant decrease in survival rates of Gf2. The sub-lethal investigations showed that Ag+ at 1 and 3 µg.L-1 and AgNPs 23 nm at 3 µg.L-1 significantly ($P<0.05$) decreased osmoregulation and locomotor activity. AgNPs 20 nm and AgNPs 27 nm had no effects on any of the studied responses. In addition to highlighting the potential of *G. fossarum* as a model organism in nanotoxicology, this study illustrates that AgNPs act at very low concentrations and their toxicity may be dependent on their physico-chemical properties.

2806 Evaluation of the Adjuvant Effects of Transcutaneously Exposed Silver Nanoparticles with Different Size in Mouse Model

Y.-M. Cho[3], Y. Mizuta[3], T. Toyoda[3], J.-i. Akagi[3], T. Hirata[1,3], R. Adachi[3], Y. Kimura[3], T. Nishimaki-Mogami[3] and K. Ogawa[3]. *[1]Department of Pharmacology, School of Pharmacy, Showa University, Tokyo, Japan; [2]Division of Biochemistry, National Institute of Health Sciences, Tokyo, Japan and [3]Division of Pathology, National Institute of Health Sciences, Tokyo, Japan.*

Silver nanoparticles (AgNPs) are, largely due to their antimicrobial properties, the most commonly used in consumer products such as cosmetics, clothing, household products, room sprays and even in food products. Although a human exposure via dermal as well as oral routes has been rapidly increased by large amount of manufacture and utilization, there are few reports on the immunotoxicity of transcutaneously exposed AgNPs. We investigated immunological and histopathological changes of mice transcutaneously sensitized with AgNPs. Citrate coated AgNPs of different primary sized (10, 60 and 100 nm in diameter) at a dose of 49 µg in 2 mM citrate buffer (vehicle) with 100 µg ovalbumin (OVA) was applied in skin patches to the left flanks of 8-week old BALB/c female mice 3 days a week for 4 weeks, and then an immune response was evoked with 1 or 5 mg of OVA given via i.p. or i.g. routes, respectively, and rectal temperatures, scores of anaphylactic responses, plasma histamine levels and histopathological changes were determined. In the OVA + AgNPs groups evocated via i.p. injection, increased IgG1 and IgE levels after sensitization and decreased rectal temperature, with augmented anaphylaxis scores and plasma histamine concentrations after evocation were noted, Ki67-positive germinal center in lymph nodes being observed more frequently than in the vehicle group. In the OVA + AgNPs groups evocated via i.g. injection, increased IgG1 and IgE levels were observed. In the both groups, no significant differences in any parameters between OVA and OVA + AgNPs groups were observed. Although this model is useful for evaluation of sensitization and anaphylaxis reaction, transdermalexposure to AgNPs is not sufficient to activate key immune pathways necessary for sensitizing mice for immediate hypersensitivity reactions in the present model.

2807 Abstract Withdrawn by Author.

2808 Pulmonary Toxicity and Mortality in Mice after Intratracheal Instillation of ZnO Nanoparticles in Three Laboratories

N. R. Jacobsen[1], T. Stoeger[5], S. van den Brule[6], A. T. Saber[1], A. Beyerle[5], G. Vietti[6], A. Mortensen[7], J. Szarek[4], A. Kermanizadeh[3], A. Banerjee[2], N. Ercal[2], U. Vogel[1], H. Wallin[1] and P. Møller[3]. *[1]Danish Centre for Nanosafety, National Research Centre for the Working Environment, Copenhagen, Denmark; [2]Department of Chemistry, Missouri University of Science and Technology, Rolla, MO; [3]Department of Public Health, University of Copenhagen, Copenhagen, Denmark; [4]Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland; [5]Institute of Lung Biology and Disease, Helmholtz Zentrum München, Munich, Germany; [6]Louvain Centre for Toxicology and Applied Pharmacology, Université catholique de Louvain, Brussels, Belgium and [7]National Food Institute, Technical University of Denmark, Søborg, Denmark.*

Inhalation is the main pathway of ZnO exposure in the occupational environment but few studies have addressed toxic effects of pulmonary exposure to ZnO nanoparticles (NP). Here we present results from studies in three laboratories of pulmonary intratracheal instillation exposure and toxicity of ZnO NP in mice. Three different ZnO NP were used; the particle size and BET surface area ranged between 12–94 nm and 13–60 m2/g for the three materials. Two of the three studies were prematurely terminated because interim results unexpectedly showed severe toxicity. In the third study a high bolus doses of ZnO NP (25–100 µg (1.4-5.4 mg/kg) were clearly associated with a dose dependent mortality in the mice. Lower doses (from 6 µg (0.3 mg/kg) elicited acute toxicity in terms of reduced weight gain, desquamation of epithelial cells with concomitantly increased barrier permeability of the alveolar/ blood as well as DNA damage. Oxidative stress was shown in terms of a strongly increased lipid peroxidation and reduced glutathione in the pulmonary tissue. Blood parameters showed increased neutrophils, red blood cells, hematocrit, platelets and more. Two months post-exposure revealed no obvious toxicity for 12.5 and 25 µg/mouse using a range of parameters. However, mice that survived a high dose (50 µg; 2.7 mg/kg) had an increased pulmonary collagen accumulation (fibrosis) at a similar level as a high bolus dose of crystalline silica. The recovery from these end-points appeared dose-dependent. The results indicate that alveolar deposition of ZnO NP may cause significant adverse health effects.

2809 Gene Expression Change in the Frontal Cortex with Perivascular Damage Induced by Prenatal Exposure to Carbon Black Nanoparticle in Mice

M. Umezawa[3], A. Onoda[1,3] and K. Takeda[3]. *[1]JSPS Research Fellow, Tokyo, Japan; [2]Department of Hygienic Chemistry, Graduate School of Pharmaceutical Sciences, Tokyo University of Science, Noda, Chiba, Japan and [3]The Center for Environmental Health Science for the Next Generation, Tokyo University of Science, Noda, Chiba, Japan*. Sponsor: K. Nohara.

Objective: Prenatal exposure to carbon black nanoparticle (CB-NP) degenerates the brain perivascular macrophages (PVMs) and surrounding astrocytes in various regions such as frontal cortex [*1]. To understand the detail of the effect of CB-NP on the central nervous system function of mouse offspring, the profile of mRNA expression change in the frontal cortex induced by prenatal CB-NP exposure was investigated by microarray technique. Methods: Pregnant ICR mice were treated with suspension of CB-NP (0, 2.9, 15, or 73 µg/kg/time) by intranasal instillation on gestational days 5 and 9. Brains were collected from 6-week-old male offspring mice, and the frontal cortex was dissected and provided into analysis with SurePrint G3 Mouse GE 8×60K microarray (Agilent Technologies). The genes differentially expressed by prenatal CB-NP exposure was subjected to functional analysis by gene annotation with Gene Ontology as previously described [*2]. Brains were also analyzed by Periodic Acid Schiff (PAS) and glial fibrillary acidic protein (GFAP) double staining. Results: Total of 268 genes showed >2-fold dysregulation in the high-dose (73 µg/kg/time)/control groups comparison with dose dependency. The largest GO were angiogenesis (9 genes), iron ion binding (6 genes), metallopeptidase activity (6 genes), and positive regulation of cell migration (6 genes). Patterning of blood vessels, positive regulation of cell migration, chemotaxis, positive regulation of epithelial cell proliferation, and growth factor binding were also highly enriched. Few GFAP-positive astrocytes were observed in the grey matter in the 0 and 2.9 µg/kg/time groups, while many GFAP-positive astrocytes were detected in the 15 and 73 µg/kg/time groups. GFAP-positive astrocytic endfeet were detected at a site attached to PVM with enlarged PAS-positive granules, which are often observed in mild pathological and aging conditions. Conclusion: The present study suggests that dysregulation of angiogenesis, cell migration or proliferation, chemotaxis, and growth factor functions may be related to chronic perivascular damage induced by prenatal CB-NP exposure. References: [*1] Onoda et al. PLoS One 9: e94336 (2014); [*2] Umezawa et al. Part Fibre Toxicol 10: 64 (2013)

2810 Surface Modifications of Silica Nanoparticles Affect Their Uptake by the Cells and Subsequent Pulmonary Toxicity

S. Vranic[3,4,5], S. Ichihara[2], W. Wu[3], E. Watanabe[4], A. Hayashida[4], Y. Osada[4], T. Sakurai[4], S. Boland[6], L. Tran[1] and G. Ichihara[3,4]. *[1]Institute of Occupational Medicine, Edinburgh, United Kingdom; [2]Mie University, Tsu, Japan; [3]Nagoya University Graduate School of Medicine, Nagoya, Japan; [4]Tokyo University of Science, Noda, Japan; [5]University of Manchester, Manchester, United Kingdom and [6]University of Paris VII Diderot, Paris, France.*

It is necessary to clarify the relationship between specific properties of nanomaterial (NM) and induced biological effect, as this could lead to the development of NM "safe by design". Silica nanoparticles (SiO2 NPs) are one of the most exploited NPs nowadays. Due to their presence in the cosmetic additives, drugs and even food, a chance of exposure to these NPs is increasing, raising the question of their potential cytotoxicity. In this study we examined how different surface modifications of SiO2 NPs affect their biological interactions. SiO2 NPs of 25 nm in diameter were surface-modified by attaching amino groups, carboxyl groups or hydroxyl groups rendering them either positively, negatively or neutrally charged, respectively. Male C57BL/6J mice, 8 weeks old were exposed to 2 mg/kg or 10 mg/kg SiO2 NPs by pharyngeal aspiration. After 24 hours, the bronchoalveolar lavage fluid (BALF) was collected to determine the total and differential cell count, protein levels and cytokine secretion. Cells taking up SiO2 NPs were observed using confocal microscope. Positively charged SiO2 NPs induced the increase in the number of total cells, neutrophils, total proteins, TNF-a and MIP-2. On the other hand, the negatively charged SiO2 NPs didn't induce any change in the levels of these parameters. The hydroxyl-modified SiO2NPs increased the number of total cells, neutrophils, total protein, TNF-a and MIP-2 only after treatment with the high dose of NPs (10 mg/kg). We could also observe the cells taking up SiO2 NPs by the confocal microscope. We concluded that induction of the inflammatory response in the murine lung was dependent on the surface modifications of NPs.

2811 Single-Walled Carbon Nanotubes Suppress the Pulmonary Immune Response and Increase Viral Infectivity of Exposed Mice

H. Chen, X. Zheng, J. Nicholas, J. C. Loeb, J. H. Bisesi, S. Robinson, J. A. Lednicky and T. Sabo-Attwood. *Department of Environmental and Global Health, University of Florida, Gainesville, FL*. Sponsor: T. Sabo-Attwood.

Background: Extensive application of nanomaterials has raised concern regarding their potential health impacts. Many toxicological studies have focused on injury caused by single nanoparticle exposures but few have investigated how such exposures can impact a host's immune response to pathogen challenges. Our previous work has shown that pre-exposure of lung cells to single-walled carbon nanotubes (SWNT) modulates expression of several inflammatory and anti-viral genes in concert with increased viral titers following subsequent exposure to influenza virus (IAV). To further investigate if this observation would occur *in vivo*, we performed experiments in a mouse model of dual SWNT-IAV exposure. Methods: Male mice were randomly assigned into control, SWNT, IAV, and SWNT+IAV groups and exposed to either 20 ug of SWNT or control vehicle (pluronic) by pharyngeal aspiration. On day 3, animals were then given 3.4×10^4 IAV or PBS by intranasal instillation. All animals were euthanized on day 7 and whole lung images were captured using near-infrared fluorescence (NIRF) to track and localize SWNT in tissues. Viral titer, immune cells in the bronchoalveolar lavage fluid (BALF), and expression of several immune genes in lung tissues were quantified. Results: NIRF images suggest that SWNT remain in the lung over the course of the experiment. Results from viral titer assays show a 64-fold increase in the lungs of SWNT+IAV exposed mice compared to the IAV only group. Quantitation of immune cells in BALF indicated an increase in lymphocytes in the SWNT group, an increase in neutrophils in IAV group and a combined additive profile in the SWNT+IAV group. Lastly, all IAV exposed animals showed increased expression of IFNβ1, IFIT2,

IFIT3, CCL5, IL8 genes and interestingly, their expression was repressed in the presence of SWNT (SWNT+ IAV group) with the greatest effect occurring for IFNβ1. Conclusion: These results demonstrate that SWNT can suppress the immune response *in vivo* that may lead to increased susceptibility to viral infections.

2812 Adverse Effect of Titanium Dioxide Nanoparticle on Testicular Function

N. Miura and K. Ohtani. *Japan National Institute of Occupational and Health, Kawasaki, Japan.* Sponsor: N. Miura.

Nanosized titanium dioxide (TiO2) is manufactured worldwide in large quantities for use in a broad range of applications such as cosmetics, pigments, and antibacterial agent. Recent works indicate that nano-sized paticles (and their chemical compounds) possess testicular function toxicity. In this study, we examined the effect of TiO2 on testicular function in mice. TiO2 (Aeroxide P25; TiO2-P25) were dispersed in disodium phosphate solution (DSP) by sonication. Male C57BL/6J mice were injected TiO2 intravenously at 0, 1, 2 or 10 mg/kg body weight, once per week, for 4 weeks. Mice were sacrificed 3 days and 90 days after the last treatment. Although TiO2-P25 significantly reduced sperm numbers in addition to sperm motility at 3 days after the last injection, TiO2-P25 had no effect at 90 days after. Plasma testosterone concentrations at day three were not different between control group and TiO2-P25 injection groups. Further, we observed no significant histological change in testis among control group and TiO2-P25 injection groups. Our results indicated that nanosized TiO2 possesses adverse effect on testicular function in mice.

2813 *In Vitro* Cytotoxicity and Genotoxicity Studies of Titanium Dioxide (TiO2) Nanoparticles

M. A. Karakaya, O. C. Ulker, A. Ustundag, C. O. Yalcin and Y. Duydu. *Department of Toxicology, Ankara University, Faculty of Pharmacy, Ankara, Turkey.* Sponsor: A. E. Karakaya.

There are increasing safety concerns about the development and abundant use of nanoparticles. Titanium dioxide nanoparticles (TiO2-NPs) are increasingly being used in pharmaceuticals, cosmetics and foods due to the unique properties derived from their small sizes. TiO2-NPs can cause inflammation, tissue damage, fibrosis, and tumors and they are possibly carcinogenic to humans. Because cancer is a disease involving mutation, there are a large number of studies on the genotoxicity of TiO2-NPs. However, the results of these studies are not compatible with each other. In this study we investigated the cytotoxic and genotoxic effects of three different nanosize (20 nm, <100 nm and <150 nm) of TiO2 NPs by using Neutral Red Uptake (NRU) assay, *in vitro* comet assay in human keratinocyte cell line HaCaT and bacterial reverse mutation test (Ames test) with Salmonella typhimurium TA98, TAMix. The tested concentrations of TiO2 NPs sizes (5, 10, 50 and 100 µg/mL for each particle sizes) of 20 nm, <100 nm and <150 nm by using NRU assay has no cytotoxic effect. However at 20 nm particle size of TiO2 NP's IC20 was calculated at 100 µg/mL concentration. Comet scores obtained from TiO2 NPs exposed HaCaT cells showed that 20 nm TiO2 NPs induced DNA damage in all tested concentrations of 5, 10, 50, 100 µg/mL. Ames test results demonstrate that three different nanosize of TiO2 NPs at 6 different concentrations did not create a frameshift or base-pair substitution type mutation. In conclusion, according to our results, especially in high concentrations and small particle size of TiO2 NP's show genotoxic effect in HaCaT *in vitro*. This study has been supported by The Scientific and Technological Research Council of Turkey (TUBİTAK) (Project no: 113S229).

2814 Toxicity of Differently Charged and Sized Silver Nanoparticles to Human Alveolar Epithelial Cells A549 and Yeast *Saccharomyces cerevisiae* BY4741: Particle-Cell Interactions

K. Kasemets[1,2], A. Kahru[2] and P. Mantecca[1]. [1]*Department of Earth and Environmental Sciences, Research Center POLARIS, University of Milano Bicocca, Milano, Italy and* [2]*Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia.* Sponsor: K. Savolainen.

Of the existing nanomaterials, silver nanoparticles (AgNPs) have the highest degree of commercialization, especially in antimicrobial applications. However, the toxicity mechanisms of differently sized, shaped and charged AgNPs to the different types of the cells are still largely unknown and remain to be explored. The current study focuses on the size and charge-dependent toxicity of silver nanoparticles (AgNPs) to the different types of eukaryotic cells with different cell surface properties - human alveolar epithelial cells A549 and yeast *Saccharomyces cerevisiae* BY4741. Yeast, differently from the mammalian cells have the rigid cell wall consisting of B-glucans, chitin, mannans and mannoproteins and similarly to mammalian cells have also the endocytosis allowing internalizing the proteins and small particles. AgNPs with different size, 10 nm and 80 nm, and coating, citrate or branched-PEI (nanoComposix) leading to negative or positive surface charge, respectively, were assessed. AgNO3 was used as ionic control. Confocal, scanning and transmission electron microscopy (SEM, TEM) analysis was applied to visualize the nano-bio interactions (adsorption and internalization). UV-vis absorption spectroscopy was used to evaluate the stability of AgNPs in the culture medium. Confocal analysis revealed that all the studied AgNPs were internalized by the mammalian as well as by yeast cells after 24-h exposure in the cell culture medium or deionized water, respectively. Positively and not negatively charged AgNPs adsorbed onto the cell surface and were more toxic than negatively charged NPs suggesting that the toxicity of silver NPs is crucially driven by the particle-cell interactions. Physicochemical interactions at the nano-bio interface may lead to the enhanced ionic stress, membrane damage and oxidative injury of the cell. Specific mechanism is under the investigation. This work was supported by Fondazione Cariplo (OverNanotox 2013-0987), IUT 23-5 and ETF9001.

2815 Abstract Withdrawn by Author.

2816 Effects of Nano-Sized Titanium Dioxide on the Adhesion of Monocytes to Endothelial Cells

S. Ichihara[1], Y. Suzuki[1], E. Watanabe[2], Y. Osada[2], A. Hayashida[2], T. Furutani[1], K. Izuoka[1] and G. Ichihara[2]. [1]*Mie University, Tsu, Japan and* [2]*Tokyo University of Science, Noda, Japan.*

Background: Given that the recent explosion in the field of nanotechnology provides promising potential applications of manufactured nanomaterials, information about safety and potential hazards of nanoparticles is urgently needed. Nanoscale titanium dioxide (TiO2) is one of the most commonly manufactured nanoparticles. However, the effects of exposure to the nanoparticles on the cardiovascular system remain unknown. The present study investigated the effects of nano-sized TiO2 on the adhesion of monocytes to endothelial cells, which are essential processes in atherosclerogenesis, by the differences in the crystal structure and characteristics of the surface of nanoparticles. Methods and results: Human umbilical vein endothelial cells (HUVECs) and human monocytic leukemia cells (THP-1) were cultured and exposed to anatase, rutile hydrophobic, and rutile hydrophilic TiO2 nanoparticles. The MTS assay showed that incubation of HUVECs in the presence of all particles at 100 µg/ml significantly reduced cell viability. Cell viability was significantly decreased after exposure to 75 µg/ml of anatase TiO2 particles. The 3-hour exposure to anatase TiO2 and rutile hydrophobic TiO2 particles increased the level of intercellular adhesion molecule-1 (ICAM-1) in HUVECs. Exposure to anatase TiO2 particles also upregulated the expression of lymphocyte function-associated antigen-1 (LFA-1) and tissue necrosis factor alpha (TNF-α) in THP-1 monocytes. Conclusion: The present study demonstrated that exposure to anatase TiO2 particles increased the adhesion molecules, which induced adhesion of THP-1 cells to HUVECs.

2817 Maternal Engineered Nanomaterial Inhalation During Gestation Alters the Fetal Transcriptome

C. E. Nichols[2], J. M. Hollander[2], K. Engels[2], C. R. McBride[2], J. Yi[2], D. L. Shepherd[2], A. B. Abukabda[2], P. A. Stapleton[2] and T. R. Nurkiewicz[2]. [1]*Exercise Physiology, West Virginia University, Morgantown, WV and* [2]*Physiology and Pharmacology, West Virginia University, Morgantown, WV.*

Engineered nanomaterial (ENM) exposures have become commonplace, and whether unintentional or intentional, produce untoward biological effects. We have reported ENM inhalation such as titanium dioxide (nano-TiO2) impairs systemic microvascular reactivity, associated with oxidative stress dependent mechanisms in the microvascular wall. Further, ENM exposure during pregnancy, not only decreases maternal vascular reactivity and induces mitochondrial dysfunction but also decreases fetal vascular reactivity, individual pup mass and litter number. Collectively, this indicates that ENM sensitivity and microvascular dysfunction may begin *in utero* and persist during development, which could be associated with altered transcriptomic profiles after maternal ENM exposure. The purpose of this study was to identify mRNAs

that were differentially expressed as a result of the hostile gestational environment initiated by maternal ENM inhalation. Pregnant rats were exposed to nano-TiO2 aerosols (154±0.5 nm, 10.5±0.5 mg/m3, 5 hours) in the second half of gestation for 4 days (final pulmonary deposition, 16.4±0.8 µg). Following harvest on gestational day 20, RNA from fetal hearts underwent RNA-seq followed by Ingenuity Pathway Analysis (IPA). IPA identified a number of dysregulated processes including decreased oxidative phosphorylation (56 markers (mks), P<0.01), and increased mitochondrial dysfunction (92 mks, P<0.01) following ENM exposure. Further, cardiovascular development and function was significantly dysregulated following ENM exposure (1043 mks, P<0.01). Enhanced cardiotoxicity was represented largely by increases in markers of hypertrophy (227 mks, P<0.01), failure (149 mks, P<0.01), and necrosis (149 mks, P<0.01). Considered together, these data support our hypothesis that maternal ENM exposure affects fetal cardiovascular health by impacting the transcriptome, possibly predisposing offspring to future adverse cardiovascular outcomes. Future studies are planned to realize the full physiological relevance of these findings by determining the participation and stability of epigenetic modifications through DNA methylation analysis and downstream protein/microvascular evaluation. NIH ES015022; ES024783.

2818 Instillation of Different Aspect Ratios of Carboxylated MWCNT Yield Similar Vascular Contraction and Relaxation Responses in Male and Female Sprague Dawley Rats

C. J. Wingard[2], J. Odom[2], N. A. Holland[2], T. R. Fennell[1] and J. Volkan[2]. [1]*RTI International, Research Triangle Park, NC and* [2]*Physiology, East Carolina University, Greenville, NC.*

Rationale: Modified multiwalled carbon nanotubes (MWCNTs) are being increasingly designed for consumer and biomedical applications. There is an ongoing discussion as to the potential for the aspect ratio to drive apparent toxicological effect of MWCNTs. We were interested if MWCNT aspect ratio of a carboxylated form of MWCNT would result in altered vascular responses. Methods: We exposed male, non-pregnant female and pregnant (gestational day 17–19) Sprague Dawley rats to 1 mg/kg MWCNTs suspended in 5% Infasurf surfactant/saline by intratracheal instillation. Three forms of carboxylated MWCNT with different diameters and lengths were provided by the NIEHS Centers for Nanotechnology and Health Implication Research Consortium as. Vasoactive responses of aorta, coronary and uterine artery were studied by wire myography 1 and 7 day post instillation. Results: The aortic vessel responses showed only modest depression of the constrictor responses in both sexes; however sensitivity to the constrictor agents was gender and MWCNT dependent with tendency to desensitization from 1–7 day post instillation. The relaxation with ACH and SNP were slightly augmented and increased at 7 days. Coronary vessel responses were depressed with increased sensitivity to the agonist in both sexes. Uterine artery response from non-pregnant females showed the greatest changes following instillation of all MWCNTs with an increase in stress generation and desensitization to the agonist PE, but opposite response for ANG II. The relaxation responses to ACH and SNP were also augmented with MWCNT exposure. Conclusions: These results suggest a greater susceptibility of the uterine vasculature to pulmonary instillation of MWCNT than coronary or Aorta and that changes in vessel responses to MWCNT could be observed 7 days posts instillation. There appears to be limited and no definable responses attributed to the physical characteristics of the MWCNT instilled. In general the short narrow MWCNT were least impactful on vascular response changes while either being wide and short or narrow or long had greater impacts on vascular responses. Supported in part by NIEHS U19ES019525 and East Carolina University.

2819a Effect to Titanium Dioxide Food Grade (E171) in a Colorectal Carcinogenesis Murine Model

L. G. Garduño-Balderas, I. M. Urrutia-Ortega and Y. I. Chirino. *UNAM, Estado de Mexico, Mexico.*

Colorectal cancer (CRC) is the second and third most common cancer worldwide in women and men, respectively and its etiology is associated to several factors including hereditary and lifestyle, which includes diet habits. Twenty percent of CRC cases are associated to western style diets and some food additives could be a risk for the development of CRC. In this work we investigated the effect of titanium dioxide, named in food industry as E171 on formation of tumor in CRC mice model using a dose of 5 mg/kgbw/ig in male mice BALB/c during 5 days/10 weeks. CRC were induced with a single azoxymethane (AOM) injection (12.5 mg/kgbw/ip) and three cycles of 2% dextran sulfate sodium (DSS) in water ad libitum, following the scheme of a week with DSS in water and two weeks with water without DSS repeated 3 times. Mice were divided in 4 groups; control group (Ct), E171 group (E171), CRC group (CRC) and CRC+E171 group (CRC+E171). Histological analysis showed that E171 group had an increase of crypts size revisted with hyperplastic epithelium with slight dysplastic changes. CRC group had polypid lesions constituted by well conformed glands revisted by dysplastic epithelium corresponding to well differentiate adenocarcinomas and CRC+E171 group showed well-preserved glands with some areas of neoplastic cells with atypia, corresponding to mildly differentiated adenocarcinoma. Moreover, E171, CRC and CRC+E171 groups had a decrease in goblet cells of 51.5%, 57.2% and 71.1%, respectively compared with the control group. CRC group presented 7 tumors on average in the distal colon while CRC+E171 group presented an increase in colon tumor formation by 300%. The loss of goblet cells might be the way that E171 exacerbates tumors formation since goblet cells are responsible to secrete the colon protective mucus layer.

2819 Impact of Instilled MWCNT Aspect Ratio on Coronary Artery Responses and Cardiac Ischemia-Reperfusion Injury in Male Sprague Dawley Rats

N. A. Holland[2], J. Volkan[2], J. Odom[2], T. R. Fennell[1] and C. J. Wingard[2]. [1]*RTI International, Research Triangle Park, NC and* [2]*Physiology, East Carolina University, Greenville, NC.*

Rationale: In recent years the potential uses of engineered nanomaterials have rapidly expanded in the fields of biomedical technology and consumer manufacturing. Multi-walled carbon nanotubes (MWCNTs) have garnered much interest due to their physiochemical properties. However, concerns regarding the potential toxicity resulting from pulmonary exposure to MWCNTs have been raised. Negative cardiovascular consequences have been demonstrated following exposure to nanomaterials including MWCNTs. Herein, we report on the impact of different aspect ratios of MWCNTs on coronary vascular reactivity and cardiac ischemia/reperfusion (I/R) injury following pulmonary instillation. Methods: Male Sprague-Dawley rats were instilled with 1mg/kg of carboxylated MWCNTs suspended in 5% Infrasurf surfactant/saline. Twenty-four hours following intratracheal instillation of one of three forms of a carboxylated MWCNTs with different aspect ratios or 5% Infrasurf surfactant/saline vehicle rats underwent temporary ligation of the left anterior descending coronary artery or assessment of coronary artery reactivity by wire myography. Results: Exposure to all forms of MWCNTs resulted in depressed coronary stress generation in response to serotonin compared to vehicle or naïve, as well as impaired vascular relaxation in response to acetylcholine and sodium nitroprusside. Instillation of MWCNT also resulted in expansion of cardiac I/R injury independent of aspect ratio compared to vehicle or naïve 24 hours following instillation. Conclusions: Instillation of MWCNTs results in alterations of coronary artery reactivity, which may contribute to expansion of cardiac I/R injury. Cardiovascular responses are independent of MWCNT aspect ratio, reflecting an overall deleterious impact on coronary artery function and myocardial injury responses following pulmonary responses to MWCNTs. Supported in part by NIEHS U19ES019525 and East Carolina University.

2820 Nano-Titanium Dioxide Exposure Differentially Affects Segmental Vascular Reactivity

A. B. Abukabda, P. A. Stapleton and T. R. Nurkiewicz. *Physiology and Pharmacology, West Virginia University, Morgantown, WV.*

Background: Engineered nanomaterials (ENM) are anthropogenic materials with at least one dimension <100 nm. ENM exposure in industrial and domestic scenarios has been associated with cardiovascular dysfunction, with disparate results being reported depending on the vascular tissue studied. In order to determine the most sensitive levels of the vasculature where these effects manifest, a serial assessment across distinct divisions of the vascular tree was performed. Therefore, the aim of this study was to determine the impact of ENM exposure on vascular reactivity in regionally discrete segments. We hypothesize that acute nano-TiO2 exposure differentially affects reactivity with a more robust impairment in the microcirculation. Methods: Wire myography was used to evaluate active tension generation in the thoracic aorta, the femoral artery and 3rd order mesenteric arterioles from Sprague Dawley rats. Denudation via physical abrasion was performed to determine the influence of the endothelium following exposure. Two exposure routes were selected: tissue coincubation (30 minutes; 20, 100 and 200 µg per 250 µl of medium) and lung via intratracheal instillation [(IT; 24 hours prior; 24, 120, or 240 µg suspended in 300 µl of vehicle (saline and 5% FBS)]. Rings were preconstricted with phenylephrine (PE; 1x10-3 M) and challenged with acetylcholine (ACh) (1x10-9-1x10-4 M), an endothelium-dependent agonist, and sodium nitroprusside (SNP) (1x10-9-1x10-4 M), an endothelium-independent agonist. Results: Instillation of 240 µg nano-TiO2 did not affect aortic vascular smooth muscle responses to SNP and PE, while

sensitivity to ACh decreased by 46 ±4% compared to controls. Similarly, coincubation with 200 µg of nano-TiO2 reduced aortic responsiveness to ACh by 37 ±9%, while increasing adrenergic reactivity by 43 ±7%. Endothelial denudation produced similar responses to all agonists in both unexposed control and exposed rings. Coincubation diminished femoral artery and mesenteric arteriole sensitivity to ACh by 61 ± 7% and 94 ± 5% respectively. Instillation reduced femoral artery cholinergic response by 47 ± 6%. Conclusions: These studies provide additional evidence of endothelial dysfunction that is consistent with previous findings. Moreover, this dysfunction increases in severity moving toward the resistance vasculature, suggesting that an ENM toxicity gradient exists, and appears to be most robust at the microvascular level.

2821 Upregulation of Cell Adhesion Molecules by Doxorubicin in Rat H9c2 Myocardial Cells

Z. Jia[2], R. Chitrakar[2], P. Nallasamy[2], H. Bibi[2], K. Traore[1], H. Zhu[1] and Y. R. Li[1]. *[1]Campbell University School of Osteopathic Medicine, Buies Creek, NC and [2]Biology, University of North Carolina at Greensboro, Greensboro, NC.*

Doxorubicin (Dox) is one of the most important anticancer drugs for the treatment of a wide variety of cancers. The downside associated with the use of Dox in the clinic is the high risk of irreversible cardiomyopathy and cardiotoxicity, but the precise mechanisms remain to be defined. In this study, we used qRT-PCR to examine the expression of vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-Selectin following Dox treatment in rat H9c2 cardiomyocytes. Our results showed that Dox, at a clinically relevant plasma concentration, significantly increased the expression of adhesion molecule VCAM-1, ICAM-1, and E-Selectin in rat H9c2 cardiomyocytes. . Further, Dox induced transcriptional activities of nuclear factor-kappa B (NF-kB) and antioxidant response element (ARE)in endothelial cells. These results provide the direct evidence that the expression of adhesion molecules and the consequent inflammation could be a critical player in Dox-induced cardiomyopathy and cardiotoxicity.

2822 Establishment of Cell Based Model for Studying Human Angiotensinogen Gene Regulation After Xenobiotics

C. M. Menezes[1] and R. A. Ansari[2]. *[1]Pharmaceutical Department, Federal University of Bahia (UFBA), Tv. Barão de Jeremoabo, Ondina, Salvador, Bahia, Brazil and [2]Pharmaceutical Sciences, Nova Southeastern University, Fort Lauderdale, FL.*

Angiotensinogen is a liver secreted protein which plays a significant role in blood pressure regulation and mediate fibrogenic transformation of liver after hepatotoxicants mediated liver injury. The regulation of human angiotensinogen differs from mouse and rats. Using the human angiotensinogen transgenic animals for studying its regulation is costly. Alternatively, we have demonstrated earlier that human hepatocytes can be used for studying regulation of human angiotensinogen after treatment with ethanol. Since hepatotoxicants' exposure induce immune response due to liver injury, we have earlier studied the effects of interleukins (IL-1β, and IL-6) and PMA (phorbol 12-myristate 13-acetate) ester on the secretions of AGT from HepG2 and Huh7 cells. Treatment of these hepatocytes resulted into increased secretion in media after treatment with IL-1β, IL-6 and PMA after 6 and 12 hrs. The effects of these treatments on the cellular level of AGT were also determined to correlate the secretions with cellular AGT level. It was observed that cells possessed 3 forms of AGT. The levels of matured AGT in cells were not increased but rather a decrease was observed in treated hepatocytes. There were two lesser size, unmatured1 and further less size, unmatured 2 AGT. The unmatured form of AGT (unmatured1 and unmatured 2) were increased in treated groups in both cells compared to control. It is concluded that these cells can serve as cell based-model instead of transgenic for studying the effects of toxicants on angiotensinogen gene regulation.

2823 Oleic Acid Exposure of Cultured Endothelial Cells Alters Lipid Mediator Production

V. Bass[2] and M. Madden[1]. *[1]CRB, EPHD, NHEERL, ORD, US EPA, Chapel Hill, NC and [2]Environmental Sciences and Engineering, University of North Carolina-Chapel Hill, Chapel Hill, NC.*

Diesel, biodiesel, and other combustion sources contain free fatty acid (FFA) components capable of entering the body through particulate inhalation. FFA can also be endogenously released into circulation in response to stress. When in circulation, bioactive FFA may interact with cells lining the blood vessels, potentially inducing endothelial dysfunction. To examine the effects of FFA on vascular cells, human umbilical vein endothelial cells (HUVEC) were exposed to oleic acid (OA) with one of two vehicles, either bovine serum albumin (OA-BSA) or ethanol (OA-EtOH). Dose-dependent cytotoxicity was noted at 4hr with an increase in LDH activity at 250µM OA-BSA (25% above controls), increasing to 50% in HUVEC exposed to 1mM OA-BSA for 48hr. These observations were confirmed through dose-response testing of viability (MTT assay), which yielded a 20% reduction in viability relative to controls at 1mM OA-BSA. These data demonstrate that the threshold for direct OA-induced cytotoxicity is approximately 100µM OA-BSA at 24hr. HUVEC were also exposed to methylated-OA at similar doses, which yielded no increase in LDH release, suggesting that the carboxyl group on OA is necessary to induce cytotoxic effects. 14C-OA cellular uptake experiments showed that 80% of labeled OA-EtOH became associated with HUVEC, versus only 8% of OA-BSA at 24hr. Analysis of soluble mediator release from HUVEC showed a dose-dependent increase in prostaglandin F2α, a lipid involved in control of vascular tone, at 24hr (85% above controls) after OA-BSA exposure. RT-PCR analysis revealed increased endothelin-1 mRNA in OA-BSA exposed HUVEC. Together, these data demonstrate that circulating FFA may be capable of inducing cytotoxic effects and altering expression of mediators of vascular function. [This is an abstract of a proposed presentation and may not reflect official US EPA policy.]

2824 Baseline Cardiac Troponin I in Göttingen Minipigs

S. W. Hulet[2], N. Vincelli[1], J. Scotto[2] and D. B. Miller[2]. *[1]Leidos, Inc., Abingdon, MD and [2]US Army ECBC, Aberdeen Proving Ground, MD.*

The use of cardiac troponin I (CTnI) as an emergency medicine biomarker indicative of acute myocardial infarction is generally accepted, with the threshold typically set at the 99th percentile value for the population. Over the course of several studies we were able to collect blood samples from a large number of young adult male Gottingen minipigs to assess baseline CTnI levels. All of the animals used in these studies had a cannula surgically implanted into the right external jugular vein which was used for whole blood collection. A subset of these animals also had DSI telemetry transmitters surgically implanted. All baseline blood samples were collected after the animals had sufficient time to recover following surgery. For the first analysis, an aliquot of whole blood from each pig was split equally into lithium heparin (LiHep) tubes and K2 EDTA and subsequently analyzed for CTnI using i-Stat cartridges. A significant difference (p=0.032) was identified in the CTnI levels in samples taken from the same animals (n=48) when stored in LiHep tubes vs. K2 EDTA tubes, with the portion stored in LiHep tube reading significantly higher than the portion stored in the K2 EDTA tube. Blood samples from an additional 61 minipigs were collected solely into LiHep (total n=109) tubes and blood samples from another 20 minipigs were collected solely into K2 EDTA (total n=68) tubes and assessed for CTnI levels in the same manner. A significant difference (p=0.019) was again identified when comparing the 2 larger datasets. No significant difference was identified in baseline CTnI levels (all in LiHep tubes) in animals that had surgically implanted telemetry transmitters (n=54) when compared to animals without transmitters (n=55). Mean CTnI in the large LiHep dataset (n=109) was 0.0233 ng/dL ±0.00642 SEM (Range 0.00-0.67 ng/dL). In order to calculate the 99th percentile for the dataset a nonparametric analysis of the population was performed. In this analysis the 25th (Q1) and 75th (Q3) percentiles were calculated. The interquartile range (IQR) was calculated as Q3-Q1. The exclusion limit was calculated as Q3 + 3(IQR) with a result of 0.12 ng/dL. This analysis resulted in the exclusion of 2 data points from the data set as statistical outliers. The 99th percentile of the resultant dataset (n=107) was calculated as 0.0792 ng/dL. For comparison, the recommended 99th percentile for marker biological significance in humans for the same i-stat cartridges is 0.08 ng/dL.

2825 Effect of Vildagliptin on LXR-ABCA1/ABCG1 Cascade in Pravastatin Treated 3T3-L1 Adipocytes

A. M. Mostafa[2], N. M. Hamdy[1], H. O. El-Mesallamy[1] and S. Z. Abdel-Rahman[2]. [1]*Biochemistry Department, Faculty of Pharmacy, Ain-Shams University, Cairo, Egypt and* [2]*Obstetrics and Gynecology Department, University of Texas Medical Branch (UTMB), Galveston, TX.* Sponsor: S. Abdel-Rahman.

Reports suggested that some statins, although effective in reducing LDL-C, they were not able to achieve the goal of enhancing HDL-C. Statins reduce the expression of liver X receptor (LXR) which regulates the expression of the cholesterol transporters ABCA1 and ABCG1. A decrease in expression of these transporters can lead to decreased cholesterol efflux from peripheral tissues leading to low levels of HDL-C. While manipulating the LXR pathway may seem as a good strategy to complement the effects of statins on HDL-C, LXR synthetic ligands as T091317 showed a significant hypertriglyceridemic action that limit their use. We recently found that the anti-diabetic drug Vildaglipin stimulates LXR expression leading to increased ABCB1/ABCG1 expression which improves cholesterol efflux from adipocytes. This suggests that a combination of Vildagliptin and statin may enhance the beneficial effect of statins on HDL-C without the side effects reported with LXR agonists. Here we test the hypothesis that a combination of Vildagliptin and Pravastatin improves cholesterol efflux. Statin treated 3T3-L1 adipocytes were treated with either T091317 or vildagliptin and the expression of LXR-ABCA1/ABCG1 cascade as well as cholesterol efflux were then determined. Our preliminary data show that such treatment combination significantly induced a 2-fold increased expression ($P<0.05$) of LXR-ABCA1/ABCG1 cascade and significantly improved cholesterol efflux by 1.5-fold ($P<0.05$). Our data may explain, at least in part, the beneficial effects reported in patients receiving both medications, who show a better improvement in HDL-C levels and opens the door for the use of such approach in a larger segment of patients (Supported by an Egyptian joint supervision grant and P30 ES00676).

2826 A Kinase Inhibitor (C377) Associated with Significantly Delayed QT Prolongation and Mortality in Dogs: *In Vitro* Characterization

K. Kahlig[1], C. Li[1], J. Limberis[1], C. Neilan[2], M. Wilichinsky[2], G. Liu[1], S. Rajamani[1], A. Carey[2], C. McMahon[2], L. Patel[4], A. Chester[1] and L. A. Burns-Naas[2]. [1]*Biology, Gilead Sciences, Fremont, CA;* [2]*Drug Safety Evaluation, Gilead Sciences, Foster City, CA;* [3]*Drug Safety Evaluation, Gilead Sciences, Fremont, CA and* [4]*Medicinal Chemistry, Gilead Sciences, Seattle, WA.*

C377 is a small molecule kinase inhibitor that did not enter development due to a highly unusual cardiovascular liability. Routine dog ECG monitoring showed a delayed and progressive QT prolongation (21 hrs post-dose) with one death likely resulting from VF/TdP. A panel of *in vitro* and *ex vivo* experiments consistent with the CiPA initiative were performed to evaluate the cardiac liability. C377 (300nM) was associated with a progressive prolongation of both the monophasic AP duration (MAPD90, 2 ± 1ms @ 10min to 15 ± 5 ms @ 90min) and QT interval (2 ± 1ms @ 10min to 12 ± 1 ms @ 90min) in Langendorf-perfused, rabbit isolated hearts. This observation contrasted with dofetilide (IhERG inhibitor), which produced an immediate and stable increase in both MAPD90 and QT. A cardiac ion channel panel (acute application <5min, 22oC) indicated minimal risk (IC50 > 18μM) for inhibition of 12 channels, including hERG, hNaV1.5, hCaV1.2 and IKs. The potency for hERG inhibition (IC50 = 19μM) increased only slightly following extended compound application (20min, IC50 = 15 μM) or by increasing the temperature to 34oC (IC50 = 11 μM). C377 did not alter hERG trafficking in the hERG Lite assay (10μM for 16hr, 37oC, 99 ± 6% of vehicle). Human induced pluripotent stem cell cardiomyocytes (iCell cardiomyocytes2) were used in MEA field potential recordings to evaluate time dependent changes in excitability and pro-arrhythmia potential. C377 (300nM, 120nM free compound) produced a concentration and time dependent prolongation of the field potential duration relative to vehicle,109 ± 7% @ 2hr and 124 ± 7% @ 72hr. Uncoordinated (arrhythmia-like) activity was observed with extended incubation at higher concentrations (3μM for 144 hrs) and (10μM and 30μM for 72 hrs). Combining ion channel inhibition and hERG trafficking studies with MEA recordings provides a rapid framework for evaluating kinase inhibitors for acute and delayed effects on cardiomyocyte excitability and arrhythmia-like activity.

2827 Cardiotoxicity Study of the Aqueous Extract of Corn Silk in Rats

A. A. Adedapo[2], O. S. Babarinsa[2], A. A. Oyagbemi[2], A. D. Adedapo[2] and T. O. Omobowale[2]. [1]*Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria and* [2]*Veterinary Medicine, University of Ibadan, Ibadan, Nigeria;* [3]*Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria.*

The ear of corn are the silky strands which run its length and these strands are known as corn silks. Folk remedies showed that the corn silks are used as an oral antidiabetic agent in China for many years. It is also used as herbal tea in other nations of the world for the amelioration of urinary tract infection. The extract is being assessed for safety in this study using histopathological changes as well as electrocardiogram (ECG). Graded doses (200, 400 and 800 mg/kg) of aqueous CS extract were administered to rats for seven days. The fourth group which served as control received 3ml/kg dose of distilled water. On the eighth day, ECG was evaluated in ketamine/xylazine-induced anaesthesia in rats to determine changes in heart rate, P-wave duration, P-R interval, R-amplitude, QRS duration, QT interval and QTc. Hearts from the experimental animals were collected for histopathological changes. The results from the study showed that there was significant change in heart rate (groups B and C), P-wave duration (group D), QT interval (groups B, C and D) and QTc (groups B, C and D) when compared to the control group. Histology also indicated that sections of the heart showed fatty infiltration of inflammed heart and areas of moderate inflammation of the atrium and ventricle. It showed that at this condition of increased heart rate there may be increased work and oxygen demand by the heart, and as a result, there may be the development of rate related ischemia. Increased QT and QTc may also be indication of tachyarrhythmias and sudden death. It could therefore be concluded from this study that though folklore indicated that corn silk (CS) is of high medicinal value but one must be careful in using this product as medicinal agent especially in patients with compromised heart conditions.

2828 Clozapine-Associated Myocarditis and Cardiomyopathy in Auckland, New Zealand

B. Bellissima[2], A. Vara[1], C. Kenedi[1], F. Garavan[1] and M. Tingle[2]. [1]*Auckland District Health Board, Auckland, New Zealand and* [2]*Pharmacology & Clinical Pharmacology, University of Auckland, Auckland, New Zealand.*

Clozapine-associated myocarditis usually presents within the first 18 weeks of clozapine therapy and is acute in nature whereas cardiomyopathy may take months or years to manifest. Despite the fact that they are long recognized as problematic, with careful clinical management of patients required, neither the true incidence of these cardiotoxicities nor the relationships with clozapine exposure are known. Myocarditis presents with symptoms that can mimic viral illness and are detectable on laboratory investigations. Cardiomyopathy presents with general signs and symptoms that include the gradual onset of shortness of breath on exertion, swollen ankles and fatigue. The causative role of clozapine is determined after excluding alternative causes such as myocardial ischemia or alcoholic cardiomyopathy. There is a lack of standardization for the criteria used to diagnose clozapine-associated cardiotoxicity and cardiologists will sometimes make arbitrary decisions to discontinue clozapine if there is uncertainty. This deprives patients of what can be a life-changing drug. This study is a series of clinical investigations to determine both the incidence of clozapine-associated cardiotoxicity and potential risk factors in patients currently taking clozapine and in patients who have died while taking clozapine. A clinical review of patient records was conducted to determine the incidence of the development of myocarditis using recommended ADHB criteria. Approximately 4% of patients initiating clozapine were diagnosed with myocarditis. However, more patients had clozapine therapy stopped due to increases in laboratory markers for cardiotoxicity, although myocarditis was not recorded as a diagnosis. The Coronial autopsy database in ADHB was searched between the years 2001–2015 using the word search parameters cardiomyopathy, myocarditis and clozapine. Each autopsy report was reviewed and available clinical laboratory results for clozapine levels, lipid profile, liver enzyme profile and diabetes markers were collated. Our initial data suggests that patients on clozapine die several years younger than individuals who die from cardiotoxicity with the same co-morbidities. Additionally high levels of clozapine in our cases at the time of death may be misleading and most likely represent post-mortem redistribution.

2829 A Kinase Inhibitor (C377) Associated with Significantly Delayed QT Prolongation and Mortality in Dogs: *In Vivo* Characterization

C. L. Neilan[2], M. Wilichinsky[2], A. Carey[2], K. Kahlig[2], M. Osinski[1], H. Drey[1], C. McMahon[2], L. Patel[2], E. I. Lepist[2], A. Chester[2] and L. A. Burns-Naas[2]. [1]*Covance Laboratories, Inc, Madison, WI and* [2]*Gilead Sciences, Foster City, CA.*

Kinase inhibitor (KI) C377 was precluded from consideration as a development candidate due to unexpected cardiovascular adverse events. In a 7-day dog toxicity study mortality occurred at ≥ 30 mg/kg on Days 4/5 in the absence of prodromal clinical signs or histopathology. During ECG collection on Day 5, a single animal given 100 mg/kg developed polymorphic ventricular tachycardia (probable Torsade de Pointe) and ventricular fibrillation, and expired. *In vitro* ion channel, receptor and kinase screens did not predict a cardiovascular signal for C377. IC50s for hERG and 11 other CV-related ion channels evaluated were >18µM. There was no effect on hERG trafficking. Subsequent single and 5-day repeat dose telemetry studies confirmed QT prolongation at doses ≥10 mg/kg. Single doses of 30 and 100 mg/kg produced persistent prolongation (up to 31 and 111 msec, respectively) of heart rate-corrected QT, beginning at 21 hr and peaking at 67 hr postdose, and persisting for the duration of data collection (4 days). At 100 mg/kg transient increases in heart rate, diastolic and mean arterial pressure occurred at 9-43 hr postdose; increased body temp (up to 2.2°C) occurred at 11-73 hr postdose. Daily administration of 10 mg/kg for 5 days produced mild but persistent prolongation of QT (9-19 msec) beginning Day 3 and continuing through Day 5. C377-related alterations in clinical pathology endpoints were confined to animals given 100 mg/kg. The onset of QT prolongation was not associated with Cmax. There was no evidence of a metabolite-mediated effect. These data indicate that the mortality noted in dogs resulted from a unique delayed and persistent QT prolongation; which occurred at projected AUC exposure margins of ≥ 2-fold. The mechanism of the observed QT prolongation is under further evaluation in several *in vitro* cardiovascular assays in order to identify an appropriate means to screen KIs against this type of cardiovascular toxicity.

2830 The Influence of Inhaled Multi-Walled Carbon Nanotubes on Autonomic Nervous System

W. Zheng[1], W. McKinney[1], M. Kashon[1], R. Salmen[1], D. Pan[1], V. Castranova[2] and H. Kan[1,2]. [1]*NIOSH/HELD, Morgantown, WV and* [2]*Department of Pharmaceutical Sciences, West Virginia University, Morgantown, WV;* [3]*Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV.*

Heart rate and cardiovascular function are regulated by the autonomic nervous system. Heart rate variability (HRV) as a marker reflects the activity of autonomic nervous system. The prognostic significance of HRV in cardiovascular disease has been reported in clinical and epidemiological studies. The present study focused on the influence of inhaled multi-walled carbon nanotubes (MWCNTs) on autonomic nervous system by HRV analysis. Methods:Male Sprague-Dawley rats were pre-implanted with telemetry device and kept in the individual cages for recovery. At week four after device implantation, rats were exposed to MWCNTs for 5h at a concentration of 5 mg/m3. The real-time EKGs were recorded by a telemetry system at pre-exposure, during exposure, 1 day and 7 days post-exposure. HRV was measured by root mean square of successive differences (RMSSD); the standard deviation of RR interval (SDNN); the proportion of NN5 or NN10 divided by total number of NNs (pNN5 or pNN10); low frequency (LF) and high frequency (HF). Results: Exposure to MWCNTs significantly increased the percentage of differences between adjacent R-R intervals over 5 ms (pNN5) and 10 ms (pNN10) ($p<0.01$), the root mean square of the successive differences (RMSSD) ($p<0.01$), the power of low frequency (LF) ($P<0.05$) and high frequency (HF) ($p<0.01$). Conclusions: Inhalation of MWCNTs significantly altered the performance of autonomic nervous system dominated by an increased activity of the parasympathetic nervous system during the exposure. Such a transient alteration in autonomic nervous performance may raise the risk of cardiovascular events in people with pre-existing cardiovascular conditions.

2831 Subclinical Cardiovascular Effects of Short-Term Ergot Exposure in Beef Cows

V. E. Cowan[3], T. Grusie[3], A. Neumann[3], J. McKinnon[3], B. R. Blakley[3] and J. Singh[3]. [1]*Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada;* [2]*Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada and* [3]*Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada.* Sponsor: L. Weber.

Ergot alkaloids are vasoactive compounds encountered in livestock feed. Prolonged exposure to ergot can produce clinical disease in cattle marked by reduced blood flow to peripheral vasculature leading to gangrene of hooves, tail tips, and ears. However, subclinical cardiovascular effects of ergot in cattle are poorly understood. This study was conducted to characterize hemodynamic changes in three arteries in response to increasing ergot concentrations in cattle feed that were at or below permissible levels of 2000ppb. Beef cows (n=16) were randomized into four groups: control (0ppb), low (125ppb), medium (500ppb), and high (2000ppb). Diets were fed daily for one week. Control diets were fed four days before and after the experimental period. The caudal, internal iliac, and median sacral arteries were imaged daily using color flow Doppler ultrasonography to assess the following hemodynamic parameters: arterial diameter, pulsatility index, resistivity index, blood velocity, blood flow per minute, and volume per heartbeat. Data were analyzed by repeated measures statistics (SAS; $P<0.05$). The caudal artery was most responsive to ergot. Internal iliac artery hemodynamics were unaffected at these levels of ergot feeding. Intermediate effects were observed in the median sacral artery. Caudal artery diameter was reduced at 2000 ppb ($P=0.02$), corresponding to a 19% decrease. Caudal artery hemodynamics also affected by ergot included flow per minute ($P=0.006$), volume per beat ($P=0.004$), pulsatility index ($P=0.01$), and resistivity index ($P=0.01$). Ambient temperature influenced arterial diameter (CA $P<0.0001$; MSA $P<0.0001$), mean blood velocity (CA $P=0.0001$), flow per minute (CA $P<0.0001$; MSA $P=0.001$), and volume per beat (CA $P<0.0001$; MSA $P=0.0006$). Recovery to pre-ergot caudal artery diameter occurred within one week of ergot removal (104% of pre-ergot diameter). Ergot action appears to depend on the anatomical location of the artery (i.e., peripheral or visceral). In conclusion, changes in caudal artery vascular parameters represent a reliable biomarker of ergot exposure. Short-term dietary ergot exposure in cattle feed alters peripheral artery hemodynamics however, results are strongly influenced by ambient temperature.

2832 Integrated Evaluation of Cardiac Inotropes Using Echocardiography, Telemetry, and Arrhythmia Detection Methods in the Beagle Dog

S. D. Tichenor[2], H. Holzgrefe[2], D. Regalia[2], R. Kaiser[2], S. Hosking[2] and K. M. Hoagland[1]. [1]*Amgen inc., Thousand Oaks, CA and* [2]*Chales River Laboratories, Reno, NV.*

Drug-induced effects on cardiovascular (CV) function, including changes in hemodynamics, cardiac contractility, and electrophysiology (proarrhythmic potential), are common causes of attrition in drug development. We conducted an integrated CV assessment of positive (milrinone and amrinone) and negative (itraconazole) inotropic agents at doses reported to match clinical exposures. Six female beagle dogs were dosed with the reference agents and corresponding controls on separate days, one day for continuous 24-hr telemetry monitoring (jacketed external telemetry with blood pressure [BP] implants [Data Sciences International- DSI]) and one day for echocardiography (echo) data collection (2 hr postdose). Echo indices of left ventricular (LV) size and function demonstrated expected changes (percent control) including LV internal systolic diameter (17% and 50% decreases and 17% increase) as well as ejection fraction (18% and 43% increases and 20% decrease) for amrinone, milrinone, and intraconazole, respectively. Expected ECG effects (% control) following the milrinone dose included increased heart rate (38%), decreased PR interval (26%), decreased QT and heart rate (HR)-corrected QT (QTca) intervals of approximately 13%, as well as decreased mean BP and pulse pressure of 27%. Similarly, there were decreased QT/QTca intervals (5% compared to control) following the amrinone dose with no HR or BP effects. Arrhythmia detection using Data Insights Software (DSI) showed no consistent compound-related changes in ECG waveform morphology or rhythm. QA interval (QAI) was shorter with milrinone and longer with itraconazole, consistent with positive and negative inotropy, respectively, supporting use of QAI as an indirect measure of cardiac contractility. Collectively, these novel integrated inotropic data demonstrate that a nonclinical model integrating both telemetry and echocardiography on separate dosing days in a repeat dose study design can provide robust assessments of CV function.

2833 Resveratrol Protects Heart from Sepsis Likely by Modulating SERCA2a Activity

T. Bai[1,2], S. Wang[1], N. Mellen[1], Y. Zheng[2], J. Kong[2] and L. Cai[1]. [1]*Departments of Pediatrics, Kosair Children's Hospital Research Institute, Louisville, KY and* [2]*The First Hospital of Jilin University, Departments of Cardiovascular Center and Geriatric Medicine, Changchun, Jilin Province, China.* Sponsor: L. Cai.

Objective: Heart failure causes poor prognosis in sepsis. This study examines whether resveratrol protects animals from sepsis-induced heart failure by preventing sepsis-decreased SERCA2a activity. Methods: Sepsis was induced by lipopolysaccharide (LPS, 6 mg/kg) intraperitoneal injection. Four groups of mice were designated as vehicle control, resveratrol (20 mg/kg/day), LPS and LPS plus resveratrol, respectively. Cardiac function was examined by echocardiography. Cardiomyocyte contractility and calcium transient were measured in isolated adult cardiomyocytes. CM-DCFDA was used to detect cellular superoxide. Results: Cardiac function was reduced more than 50% in the LPS group while resveratrol significantly improved it. There were no differences in plasma TNF-α and troponin I levels nor in infiltration of inflammatory cells in cardiac sections. This suggests protection by resveratrol from septic heart wasn't dependent on its anti-inflammatory effect. In isolated cardiomyocytes, LPS treatment resulted in decreased contractile amplitude, longer relengthening time, and increased generation of superoxides. These damages were corrected by resveratrol treatment. Immunoblot analysis showed LPS treated mice had increased levels of endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), and 3-Nitrotyrosine (at 110 kDa where the SERCA2a is located), while the level of phosphor-phospholamban was decreased. Resveratrol treatment resulted in upregulation of phosphor-phospholamban, downregulation of eNOS and iNOS, and reduction of 3-Nitrotyrosine levels at 110 kDa. Conclusion: Our data suggests that activity of SERCA2a in sepsis may be inhibited, possible due to nitric oxidative modification and resveratrol could protect the heart from sepsis likely through modulating activity of SERCA2a.

2834 Dioxin-Like Pollutants Increase Plasma Levels of the Cardiovascular Risk Biomarker TMAO Through Upregulation of FMO3

M. Petriello[2], A. Morris[2], B. Wahlang[2], M. Sunkara[2], J. Hoffman[2] and B. Hennig[2]. [1]*University of Kentucky, Lexington, KY and* [2]*Superfund Research Center, University of Kentucky, Lexington, KY.*

Bioaccumulation of PCBs in humans is associated with an increased risk of inflammatory diseases, including diabetes, obesity and cardiovascular disease (CVD). The etiology of CVD is impacted by multiple modifiable and non-modifiable risk factors including dietary choices, genetic predisposition, and environmental exposures. However, the mechanisms linking exposure to environmental pollutants to CVD risk are largely unclear. Recent studies identified a strong link between plasma levels of nutrient-derived Trimethylamine N-oxide (TMAO) and coronary artery disease risk in humans. Previous studies showed that dioxin-like environmental pollutants can upregulate a critical enzyme responsible for TMAO formation, hepatic flavin containing monooxygenase 3 (FMO3), but a link between dioxin-like PCBs, upregulation of FMO3, and increased levels of TMAO has not been fully described. Therefore, we examined the expression levels of FMO3 in the liver in mice after oral administration of either coplanar PCB 126, PCB77, or an Aroclor mixture. PCB treated animals exhibited increased FMO3 mRNA, protein, enzymatic activity, as well as an increase in circulating levels of TMAO following acute administration of its metabolic precursors. Exposure to 5 µmole/kg PCB 126 resulted in greater than 100-fold increase in FMO3 mRNA expression and a 5-fold increase in TMAO levels. Also, liver mRNA levels of multiple genes related to metabolism, energy homeostasis, and cholesterol regulation were altered in these PCB treated mice. Taken together, these observations suggest that exposure to dioxin-like environmental pollutants may be pro-atherogenic by increasing FMO3 and TMAO levels. (Supported in part by NIH/NIEHS P42ES007380).

2835 Pilot Study Investigating the Pressor Activity of Vasopressin Infused by the Subcutaneously Implanted SMP-200 Microinfusion Pump in the Telemetered Rat

C. Bory, P. Legé, E. Chalencon, J.-P. Briffaux and S. Milano. *WIL Research Europe, Lyon, France.*

The advent of subcutaneously (SC) implantable microinfusion pumps for small lab animals has allowed a very suitable and cost effective method to deliver drugs. Combined with telemetry, such pumps are ethical and accurate alternatives to infusion in restrained or tethered conditions, allowing sensitive detection of drug induced therapeutic/adverse effects. The iPRECIO® SMP-200 pump (Primetech, Japan) is a SC implantable, transcutaneously-refillable and programmable pump for small lab animals, based on a mini-peristaltic flow infusion system. The precision of SMP-200 pump (in terms of volume delivered) was tested in 6 rats telemetered for arterial blood pressure and infused with 2 doses of Arginine Vasopressin (AVP), 20 ng/h (dose A) and 200 ng/h (dose B), both infused for 24h at 30 µL/h. One week after standard telemetry surgery, the pump catheter was implanted in the femoral vein. The pump was placed below the skin of the right flank and was initially programmed to infuse saline at 5 µL/h (for catheter patency). After maintenance infusion, the pump reservoir was emptied from remaining saline and filled with 20 ng AVP/h. The pump was then programmed for a 24h-infusion stop, at the end of which the reservoir was washed out and filled with 200 ng AVP/h. At the end of the maintenance period, actual mean infused volumes of saline agreed with programmed nominal volumes (840 µL ± 5%). At the end of the 24h-infusion of AVP at 20 ng/h, the same level of precision was observed (720 µL ± 5%) but no significant vasopressor effect was detected. AVP at 200 ng/h induced significant vasopressor activity (+20 mmHg for diastolic pressure), which started at the onset of infusion and was sustained for 24 hours. Despite the fact that, in 3 out of 6 rats, a larger deviation (± 10%) from the nominal targeted volume was noted, the precision of delivered volumes was generally satisfactory. The reason for discrepant results between actual and nominal delivered volumes during the second AVP infusion session is being investigated.

2836 The Future of Drug Safety Assessments: Artificial Learning Models in the Cardiovascular Sciences

C. A. Northcott[2], D. Vritov[1], J. Steidl-Nichols[2] and D. Eng[2]. [1]*University of Connecticut, Storrs, CT and* [2]*Business Technology, Pfizer Global R&D, Groton, CT;* [3]*Global Safety Pharmacology, Drug Safety Research and Development, Pfizer Global R&D, Groton, CT.* Sponsor: T. Brabham.

Cardiovascular (CV) safety findings contribute significantly to compound attrition, resulting in delayed or stopped clinical trials; moreover compounds have been withdrawn from the market due to CV safety liabilities. Recent advances in computer based learning models, combined with the availability of preclinical and clinical cardiovascular data, presents an opportunity to explore and leverage the use of artificial learning models for assisting with decision making, reducing late stage attrition and ultimately improving clinical outcomes. Pfizer has recently demonstrated that *in vivo* preclinical large animal CV studies (79 compounds across 10 different therapeutic areas) predict to phase 1 clinical blood pressure (BP) and heart rate (HR) findings with good specificity (true positive rate; 79%) and sensitivity (true negative rate; 78%). These data provide an ideal initial test case for a CV artificial learning model (ALM). An additional advantage of using this data set as a test case is that all studies were performed in a similar manner, reducing variability in the test set. Variables used within the ALM were dose level, free Cmax, change in blood pressure and change in heart rate observed in the preclinical dog and/or nonhuman primate study and results from Phase 1 clinical human trials. R programming language was utilized to create the neural network utilized in the ALM studies. Initial testing consisted of the development of 2 layers consisting of 20 and 15 nodes trained with a subset of the compounds, using the data from the highest no effect dose and the lowest effect dose level in the analyses. Once the neural network was trained it was then tested with 16 unique compounds, resulting in the model accurately predicting 12 of the 16 compounds (sensitivity=60%; specificity=82%). While this data was encouraging, an additional model was developed, utilizing all doses and training with 164 known observations followed by testing with 32 unique compounds. The ALM predicted with 96% specificity and 81% sensitivity, improving upon the initial network. While this first attempt at building an ALM for CV prediction is extremely simplified, the positive preliminary data demonstrate there is value in further pursuing the use of ALM for improving preclinical prediction of clinical CV outcomes.

2837 Hemodynamic Effects of Amlodipine Intravenously Infused for 6 Weeks by Implantable SMP-200 Microinfusion Pump in the Spontaneously Hypertensive (SH) Telemetered Rat

E. Chalencon, C. Bory, J.-P. Briffaux and S. Milano. *WIL Research Europe, Lyon, France.*

The recent introduction of subcutaneously (SC) implantable microinfusion pumps for small lab animals has allowed a very suitable and cost effective method to deliver drugs. Combined with telemetry, such pumps are ethical and accurate alternatives to infusion in restrained or tethered conditions, allowing sensitive detection of drug induced therapeutic/adverse effects. The iPRECIO® SMP-200 pump (Primetech, Japan) is a SC implantable, transcutaneously-refillable and programmable pump for small lab animals, based on a mini-peristaltic flow infusion system. This pump was implanted for continuous infusion for 6 weeks in 8 SH rats, telemetered for arterial blood pressure (ABP) and heart rate (HR). Saline or Amlodipine, a cardiovascular calcium channel blocker, was infused in 4 rats each. One week after standard telemetry surgery, the pump catheter was implanted in the femoral vein. The pump was placed below the skin of the right flank and was initially programmed to infuse saline at 3 µL/h (for catheter patency). At the end of this maintenance period, actual mean infused volumes of saline agreed with programmed nominal volumes (535 µL ± 6%). After maintenance infusion, the pump reservoir was emptied from remaining saline and filled with saline or Amlodipine formulation, each infused at 5 µL/h for a 7 day treatment session. Between sessions, during a 2h infusion stop, the reservoir was emptied and filled with fresh saline or Amlodipine, tested at 0.2, 1 and 2 mg/kg/h during 6 weeks. Plasma concentrations of Amlodipine were determined at the end of each treatment session. Amlodipine lowered ABP in SH rats and induced a slight and transient increase in HR, mostly during the first 24h of infusion. The precision of the volumes delivered was satisfactory. The SMP-200 pump permits elaborated infusion protocols in tetherless, free roaming and socialized rats. Combined with telemetry, this pump allows increased sensitivity and a powerful means to detect drug induced therapeutic/adverse effects, with accurate delivery of the loaded solution.

2838 Early-Life Vitamin D Deficiency Exacerbates Arrhythmia and Heart Rate Variability Responses to Smog Inhalation in Mice

K. Stratford[1], N. Haykal-Coates[2], N. Kurhanewicz[1], T. Krantz[2], C. King[2], J. Krug[2], I. Gilmour[2], A. Farraj[2] and M. Hazari[2]. [1]*University of North Carolina, Chapel Hill, NC and* [2]*Environmental Public Health Division, US EPA, Research Triangle Park, NC.*

Air pollution contributes to cardiovascular morbidity and mortality, yet the role of nutritional status in modifying susceptibility to the health effects of air pollution has not been adequately addressed. Low levels of micronutrients like vitamin D in childhood can lead to health problems in adulthood, including decrements in cardiovascular function, and might modify responsiveness to air pollution. We hypothesized that a low vitamin D diet alters the electrical and mechanical function of the heart and affects the physiological responses to smog. Mice were fed a vitamin D deficient (VDD) or normal diet (ND) after weaning. At 17 weeks of age, mice were implanted with radiotelemeters for continuous monitoring of electrocardiogram (ECG), heart rate (HR) and heart rate variability (HRV). Mice were exposed to a single exposure of atmospheric smog (1070 ug/m3 of PM2.5 and 97 ppb of ozone) or air 2 weeks later. Ventilatory function was assessed using whole-body plethysmography at specific points during the diet, before and after exposure. Tidal volume increased with age in ND mice. However, in VDD mice tidal volume decreased significantly and increased after smog when compared to air. There were no differences in the body weights or ECG parameters of any groups at any time point. Although pre-exposure HR was similar for all groups, smog exposure increased HR in ND mice; HR was further elevated by VDD. Smog significantly increased RMSSD, HF and arrhythmia in ND mice; this was further increased by VDD. These data show that apart from subtle developmental physiological effects, VDD worsens the cardiopulmonary response to smog thus highlighting the possible influence of nutritional factors in determining murine responses to air pollution. If present in man such effect modification could have significant relevance to public health. (This abstract does not reflect USEPA policy)

2839 Global Cross-Company Data-Sharing on the Housing of Non-Rodents during the Recording of Cardiovascular Telemetry Data on Toxicology Studies

H. Prior[1], A. Bottomley[1] and J. Cordes[2]. [1]*NC3Rs, London, United Kingdom and* [2]*Pfizer, Groton, CT.* Sponsor: N. Burden.

A cardiovascular assessment of new chemical entities and biologics in a non-rodent species is a regulatory requirement prior to first administration in humans. This is generally performed as a separate safety pharmacology study and/or as part of the toxicology program. An international working group and contacts within the safety pharmacology and toxicology community, including pharmaceutical and biotechnology companies and contract research organisations, has shared data on the housing of non-rodents during the recording of cardiovascular telemetry data in toxicology studies (34 respondents). This project was facilitated by the NC3Rs and the Safety Pharmacology Society. The data showed that 94% of facilities socially house their non-rodents (dogs, minipigs and non-human primates (NHP)) before and in between recording sessions on toxicology studies, however, during the cardiovascular telemetry recording 62% individually house the animals (up to 24 hours on multiple occasions during the study). Separation during recording periods may introduce unwanted stress, even when an individual is within sight/touch of another animal. The major barriers for social housing were (% respondents): damage to the jacketed equipment (70%), food consumption recording (67%), limitations of hardware (58%) and temperament of animals (50%). However, companies who socially house have found solutions to these issues and some reported that non-rodents are more cooperative, less stressed and have better baseline cardiovascular parameters (with similar data sensitivity) when socially housed. Currently, 36% and 50% of respondents socially house dogs and NHPs respectively during telemetry recording and the majority of remaining companies indicated that they would consider social housing if the barriers can be overcome. Best practice, processes and validation data shared during this project are being used to develop recommendations and increase adoption of this refinement worldwide.

2840 A Sensitive Method for Measuring ECG and Haemodynamic Parameters in Group Housed Dogs

F. Watts, B. Marshall, L. Hanson and K. Ashcroft-Hawley. *Global Safety Pharmacology, Covance Laboratories, Harrogate, United Kingdom.* Sponsor: M. Holbrook.

Compound mediated cardiovascular issues are a major cause of attrition in drug development. We have developed a model in the dog which allows group housing and the collection of data from several animals at the same time. This study describes the ability to detect small changes in ECG and haemodynamic parameters using the reference compounds sotalol, etilefrine and hydralazine. Male dogs (purpose bred pure-bred beagle dogs, obtained from Harlan, UK) surgically implanted with L11 PhysioTel® (Data Sciences International, Inc.) Model transmitters were orally administered reference compounds. Data were acquired pre-dose and for up to 24 hours post-dose. Administration of Sotalol at 3, 10 and 30 mg/kg elicited dose related increases in PR interval and QT interval, Etilefrine at 1, 3 and 10 mg/kg elicited dose related increases in blood pressure and administration of Hydralazine at 1, 3 and 10 mg/kg elicited dose related decreases in blood pressure compared to control (0.5% methylcellulose) treated animals. These effects were evident within the first 2 hours following dose administration and were maintained throughout the majority of the post-dose data collection period. In conclusion, the model which uses PhysioTel technology is capable of detecting sensitive changes in ECG and BP parameters which have been demonstrated by the use of power plots which show statistical power and the minimal detectable limit.

2841 Comparison of Left Ventricular Contractility to RR Interval Relationship in Freely-Moving Rats, Dogs and Monkeys

A. Ascah[1], M. Pouliot[1], S. Abtout[1], K. Bujold[1], R. Forster[1] and S. Authier[1,2]. [1]*CIToxLAB North America, LAVAL, QC, Canada and* [2]*Faculty of Veterinary Medicine University of Montreal, St-Hyacinthe, QC, Canada.*

Introduction: The QT-RR relationship has been extensively examined in non-clinical models but a paucity of data exist to characterize the Left Ventricular Pressure (LVP)-RR relationship across species. Methodology: LVP was continuously monitored by telemetry in conscious rats, dogs and cynomolgus monkeys following transmural telemetry catheteriza-

tion. The circadian changes to the LVP-RR relationship were evaluated in each species. Results: dP/dt+ max and contractility index as a function of heart rate showed circadian effects in all species. Only cynomolgus monkeys had statistically significant different (p<0.01) slopes during the 2 periods which was expected due to the significant circadian cycle changes documented in this species. The highest linearity of the LVP-RR relationship was observed in the rats (R2 = 0.75). Also, the variability between individual animals was the lesser in this species when compared to large animals. Discussion: Our results suggest that the conscious rat LVP model is associated with a high sensitivity owing to low variability observed in this species when compared to Beagle dogs and cynomolgus monkeys. Characterization of the LVP-RR relationship for each individual animal prior to drug administration can be valuable to enhance interpretation of ventricular contractility data.

PS 2842 Left Ventricular Pressure (LVP) and Contractility in Free-Moving Rats: The Application of Marginal Distribution Analysis

S. Abtout, K. Bujold, A. Ascah, M. Pouliot, R. Forster, E. Troncy and S. Authier. *CiToxLAB North America, Laval, QC, Canada.*

Introduction: Chronotropic effects are the most frequent cardiovascular change in drug development. Interpretation of left ventricular contractility requires correction for heart rate and circadian cycle effects. Methodology: Left ventricular pressure (LVP) was measured in conscious rats by telemetry with a transmural catheter. Pharmacological agents known for their positive inotropic (Pimobendan, Morphine and Amrinone) or negative inotropic (Atenolol and Itraconazole) properties were administered to rats and cardiovascular function was monitored for at least 24 hours after each dosing. The results were analyzed by the application of a marginal distribution curve on the contractility index and heart rate. Results: In the rat model, Pimobendan (3 to 30 kg/kg, po), Morphine (2 to 20 mg/kg, sc) and Amrinone (10 to 100 mg/kg, capsule) induced a dose dependent increases in the contractility index compared to control. Atenolol (1 to 100 mg/kg, po) decreased the contractility index in a dose-dependent manner. However, the negative inotropic effects of Itraconazole (10 to 100 mg/kg, po) on LVP were not observed in the rat telemetry model. The application of marginal distribution analysis to telemetry data at Tmax increased sensitivity compared to analysis for the entire 24 hour period. Discussion: We explored a new analysis strategy to dissociate true positive or negative inotropic pharmacological changes from physiological effect of heart rate on ventricular contractility. The application of the marginal distribution curve was considered a valuable tool in the overall interpretation of drug-induced changes in cardiac contractility.

PS 2843 DNA Damage-Inducible Transcript 4 (DDIT4) Mediates Methamphetamine-Induced Autophagy and Apoptosis Through mTOR Signaling Pathway in Cardiomyocytes

H. Wang[2], R. Chen[2,3], L. Chen[2], P. Qiu[2], J. Xu[2], E. Huang[2], C. Liu[1], Z. Lin[5] and W. Xie[2]. *[1]Guangzhou Forensic Science Institute, Guangzhou, China; [2]Forensic Medical, Southern Medical University, Guangzhou, China; [3]Forensic Medicine, Guangdong Medical University, Dongguan, China; [4]Forensic Medicine, Southern Medical University, Guangzhou, China and [5]Institute of Computational Comparative Medicine and Department of Anatomy and Physiology, Kansas State University, Manhattan, KS.*

Methamphetamine (METH) is an amphetamine-like psychostimulant that is commonly abused. Previous studies have shown that METH can induce damages to the nervous system and recent studies suggest that METH can also cause adverse and potentially lethal effects on the cardiovascular system. Recently, we demonstrated that DNA damage-inducible transcript 4 (DDIT4) regulates METH-induced neurotoxicity. However, the role of DDIT4 in METH-induced cardiotoxicity remains unknown. We hypothesized that DDIT4 maymediate METH-induced autophagy and apoptosis in cardiomyocytes. To test the hypothesis, we examined DDIT4 protein expression in cardiomyocytes and in heart tissues of rats exposed to METH with Western blotting. We also determined the effects on METH-induced autophagy and apoptosis after silencing DDIT4 expression with synthetic siRNA with or without pretreatment of a mTOR inhibitor rapamycin in cardiomyocytes using Western blot analysis, fluorescence microscopy and TUNEL staining. Our results showed that METH exposure increased DDIT4 expression and decreased phosphorylation of mTOR that was accompanied with increased autophagy and apoptosis both *in vitro* and *in vivo*. These effects were normalized after silencing DDIT4. On the other hand, rapamycin promoted METH-induced autophagy and apoptosis in DDIT4 knockdown cardiomyocytes. These results suggest that DDIT4 mediates METH-induced autophagy and apoptosis through mTOR signaling pathway in cardiomyocytes. Keywords: Methamphetamine; DDIT4 (DNA damage-inducible transcript 4); Cardiotoxicity; Autophagy; Apoptosis

PS 2844 The Estrogen Receptor Modulates STAT3/Pim-1 Signaling in a Model of Sex-Specific Pulmonary Arterial Hypertension

Y.-F. Chen[1], M. Carroll-Turpin[2], V. Y. Hebert[2], S. P. Korpela[1] and T. R. Duags[1]. *[1]Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA and [2]Department of Pharmacology, Toxicology and Neuroscience, Louisiana State University Health Sciences Center, Shreveport, LA.*

Pulmonary arterial hypertension (PAH) is a cardiovascular disease that is characterized by pulmonary arterial smooth muscle cell (PASMC) proliferation. Clinically, the incidence of PAH is almost four times higher in females compared to males, and the expression of estrogen receptor alpha (ERα) is reportedly higher in PASMC from female PAH patients. Our novel findings of 4,4'-methylenedianiline (DAPM)-induced PAH in female rats provides a likely model for studying the role of the female sex in PAH pathogenesis. Signal transducers and activators of transcription-3 (STAT3) has been studied as a potential target for cancer or PAH therapy. It has been reported in many studies that STAT3 activation plays a role in modulating the expression of a variety of proteins related to PAH pathogenesis. The expression of proto-oncogene Pim-1 has been demonstrated as an important downstream element of STAT3 signaling in PAH. In previous animal studies in our laboratory, weekly treatment with DAPM in rats caused more severe medial hyperplasia of pulmonary arterioles in females than males. To unravel the role of the estrogen receptor in DAPM-induced PAH, in these studies, we included ovariectomized females and ER antagonist ICI 182,780-treated females in our study design. Each group was treated chronically with vehicle or 25 mg/kg DAPM. In cultures of PASMCs, DAPM increased STAT3/Pim-1 signaling. However, in rats, only Pim-1 expression, and not STAT3 phosphorylation, was elevated in DAPM-treated females. From these *in vitro* and *in vivo* studies combined, we conclude that Pim-1 could serve as a target to study the role of estrogen receptor in modulating sex-specific PAH pathogenesis.

PS 2845 QT Interval Correction for Drug-Induced Changes in Body Temperature in Dogs

P. Singh, A.-I. El Amrani, F. El Amrani-Callens, S. Loriot and R. Forster. *CITOXLAB, EVREUX, France.*

Body temperature (BT) has a demonstrated influence on QT interval duration and decreased QT interval duration has been shown to be associated with increased BT in dogs. We present here an example of alteration in QT interval duration observed in a 4-week toxicology study associated with transient changes in body temperature. ECG parameters were continuously recorded on days 1 and 26 by jacketed external telemetry (JET). Each group received vehicle or test item at 10, 20 or 30 µg/kg (IV). BT was measured by rectal thermometer around 0, 0.5, 2, 4, 12 and 24 hours after dosing. Dose dependent decreases in BT were observed two hours after dosing on day 1 (38.4±0.2; 37.0±1.0 (p<0.01); 35.1±0.4 (p<0.01) and 34.1±0.6 °C (p<0.01) in vehicle and test item groups at 10, 20 and 30 µg/kg, respectively). At approximately the same time, dose-dependent increases in corrected QT interval duration (QTcV) were recorded (227±9; 265±28 (p<0.05); 285±22 (p<0.001) and 284±12 ms (p<0.001) in vehicle and test item at 10, 20 and 30 µg/kg, respectively). When QTcV was corrected for BT (QTcVcT), no effect was observed (240±11; 253±21; 252±20 and 236±9 ms in vehicle and test item at 10, 20 and 30 µg/kg, respectively). No significant changes in BT, QTcV or QTcVcT were reported on day 26. The present study demonstrates that BT can easily be monitored at appropriate time points to correct QT interval duration values for treatment related changes in BT that may occur during JET recording in regulatory toxicology studies in dogs.

2846 PACAP38 Protects Against Mitoxantrone-Induced Lethality in C57/BL6 Mice

M. K. Hughes[2], H. Xia[2], E. McIlwain[2], J. L. Maderdrut[1] and K. J. Varner[2]. [1]*Medicine, Tulane University, New Orleans, LA and* [2]*Pharmacology, LSU HSC, New Orleans, LA.*

Mitoxantrone (MXT) is an anthracenedione therapeutic that is used to treat multiple sclerosis and a variety of cancers; however, its use is limited by cardiac and other toxicities. There are no effective treatments for MXT-induced toxicity. Pituitary adenylate cyclase activating polypeptide (PACAP) is a member of the secretin/vasoactive intestinal peptide/growth hormone-releasing hormone family. PACAP protects against a number of pathological and toxicological insults. The purpose of this study was to determine whether PACAP could block MXT-induced cardiac toxicity and increase survival in mice. C57/BL6 mice were treated ip once per week for 5 weeks with saline (n = 8), MXT (3 mg/kg, n = 10) or MXT+PACAP (n = 14). PACAP38 (10µg/injection) was given 1 hr before MXT injection and 10, 24, 34, 48, 58, and 72 hr after MXT. Control and MXT groups were injected with saline on the same schedule as PACAP38. Body weight and survival were monitored. Echocardiograms were recorded before MXT administration and after 2, 3, 4, 5, and 8 weeks of treatment. After the 4th dose of MXT, the MXT mice weighed significantly less than either the MXT+PACAP or control mice. Control mice were significantly heavier than the MXT+PACAP mice. At 16 days after the 5th MXT dose, 10% of the MXT mice were alive, while 93% of the MXT+PACAP and 100% of the control mice survived. At this point, the MXT+PACAP group was divided into two groups. One group continued to receive PACAP38 (n = 7) while the other group did not (n = 6). After 20 more days, 86% of the on-going PACAP-treated group were alive, while only 14% of the PACAP withdrawal group survived. Surprisingly, echocardiography did not reveal significant differences in cardiac structure or function between the three experimental groups at any time-points. In conclusion, administering PACAP appears to protect mice from the lethal effects of MXT and, therefore, may provide a method to increase the total "life-time dose" and duration of MXT treatment. Support P30GM106392.

2847 Confocal High Content Screening of Cardiac Microtissues for the Improved Prediction of Drug Induced Structural Cardiotoxicity

S. Ravenscroft[1], C. Dilworth[2] and P. Walker[1]. [1]*Cyprotex, Cheshire, United Kingdom and* [2]*Cyprotex, Macclesfield, United Kingdom.* Sponsor: C. Strock.

Drug induced cardiotoxicity is the leading cause of attrition during drug development. Drugs can exert functional toxicities such as arrhythmia and/or morphological (structural) damage to the myocardium such as pathophysiological hypertrophy. Repeat dose drug exposures are often required for the manifestation of such toxicities, however, current preclinical *in vitro* models focus primarily on a single cell type cultured in a restrictive two-dimensional format with limited longevity. We have developed a novel iPSC derived cardiomyocyte microtissues (MTs) using a low adhesion cell suspension method which display uniform size, shape, spontaneous beating activity and longevity. As such organotypic cardiac MTs more accurately reflect the complex *in vivo* microenvironment compared with traditional 2D cell cultures. Utilising time course brightfield imaging of cardiac MTs throughout a repeat dose compound exposure setting, hypertrophy can be detected by determining MT size prior to gross cytotoxicity. MTs were exposed to 7 known pathophysiological hypertrophy inducing cardiotoxins and 12 other structural cardiotoxins (plus 2 negatives) over 14 days with 3 repeat doses (day 3, 6, 10). At day 14 confocal HCS analysis of calcium homeostasis and mitochondrial dysfunction was combined with a measure of cellular ATP, to determine structural cardiotoxicity. The lowest time course minimum effective concentration (MEC) was selected for each compound and the therapeutic index (TI) of each compound determined. With a 10-fold cut off applied, all hypertrophic cardiotoxins (except isoproterenol) induced therapeutically relevant hypertrophy. All other structural cardiotoxins including isoproterenol were correctly predicted by either calcium homeostasis and/or mitochondrial dysfunction. The use of *in vitro* cardiac MTs with multi time point confocal HCS, following repeat dosing, allows for the improved *in vitro* to *in vivo* translation of potential cardiac liabilities by novel compounds essential for the discovery of safe drugs.

2848 Integrative *In Vivo* Model for the Simultaneous Assessment of Neuro-Muscular, Cardiovascular, and Respiratory Toxicities of Oximes: The Case of Pralidoxime (2-PAM)

S. Roof, R. Hamlin and C. del Rio. *QTest Labs, Columbus, OH.*

Pralidoxime (2-PAM) is an oxime currently utilized in both the field and the clinic as a cholinesterase reactivator against nerve agent exposure. However, due to its multiple on- and off-target actions, particularly at supra-therapeutic/toxic doses, establishing specific liabilities *in vivo* remains difficult. Thus, to delineate the dose-response effects of 2-PAM on neuromuscular, cardiovascular, and respiratory systems, anesthetized (isoflurane) and mechanically-ventilated male Sprague Dawley rats were instrumented to simultaneously evaluate cardiovascular (ECG, arterial and left ventricular pressures), neuromuscular (phrenic nerve stimulated diaphragmatic force of contraction), and respiratory (pulmonary resistance/compliance) end-points *in vivo*. Animals received increasing doses of either 2-PAM (50 mg/kg IV every 20 minutes) or verapamil (0.2 mg/kg IV every 20 minutes), a known cardio-depressant calcium-channel blocker. As expected, 2-PAM elicited a progressive depression of diaphragmatic contractions, reaching complete inhibition at 150 mg/kg (-50 % at 100 mg/kg; 15.8 ± 0.3 to 8.2 ± 2.2 g), which was accompanied by minimal cardiovascular (↑BP: 4 ± 2 mmHg, and ↓HR: 33 ± 13 bpm) and/or pulmonary effects. Notably, despite diaphragmatic arrest, collapse/death were not observed likely due to assisted ventilation. In contrast, verapamil triggered progressive cardiovascular depression leading to collapse/death, despite preserved ventilation and diaphragmatic function. To determine if 2-PAM's side effects were recoverable, a separate subset of animals received IM (100 mg/kg) injections. Extended maintenance of ventilated animals (~4 hrs) provided evidence of diaphragm function recovery (presumably 2-PAM was eliminated from body). Taken together, these data 1) support diaphragmatic depression (most likely due to neuro-muscular blockade) as the primary liability of 2-PAM *in vivo*, and 2) demonstrate the feasibility of simultaneously assessing the potential neuro-muscular, cardiovascular, and respiratory toxicities of oximes pre-clinically.

2849 Novel Tissue Collection Techniques for Enhanced Detection of Nonclinical Drug Induced Vascular Injury: Integration with Off-Target Screening, Genomic, and Cardiovascular Risk Assessment Applied in a Case Study

D. Dalmas, R. Mirabile, D. Floyd, H. Thomas and K. French. *Safety Assessment, GlaxoSmithKline, King of Prussia, PA.* Sponsor: K. French.

Nonclinical drug-induced vascular injury (DIVI) is a challenging safety issue impacting various structural classes of potential pharmaceuticals which leads to attrition due to a lack of understanding of predictivity for clinical risk and lack of sensitive and/or translational biomarkers. Innovative capabilities and strategies were developed to identify risks during precandidate (PC) screening enabling educated decision making to identify the "best molecule". Novel collection techniques for rat mesenteric vasculature (MV, mesenteric roll) and dog coronary arteries (increased focus on areas containing coronary arteries from left/right aspects of heart) to enable a thorough examination of MV or heart for DIVI vs. standard collection techniques were developed along with a strategy incorporating off-target *in vitro* screening for activity known to be reflective of vascular toxicants (e.g. PDE4 inhibitor (i) activity), genomic assessment of a focused mRNA panel (VasculoTaq) in rat tissue and/or serum and standard cardiovascular (CV) functional assessments. Application of the DIVI strategy in an *in vivo* case study following oral administration for 7 days of 2 PCs kinase inhibitors of unknown mechanism were conducted to identify if either of the 2 candidates were potential vascular toxicants. The novel collection techniques increased the number of arteries present for pathological evaluation from ≈3-5 to ≈ 15-30 arteries in rat and ≈1-3 to 9 to 12/each aspect of the heart (left or right) in dog enabling a more thorough pathological examination for DIVI. Utilization of the novel strategy resulted in classification of 1 of the 2 PCs as a potential vascular toxicant based on *in vitro* evidence of PDE4i activity and alterations in mRNA expression for up to 38/51 genes on the predictive VasculoTaq panel which included alterations in TIMP1 and IL-6, genes known to be altered with PDE4-inhibitors. This enabled selection of the "best molecule" for further development. Utilization of the novel tissue collection techniques and *in vitro* evaluation for off target PDE4i activity along with genomic and CV functional assessments, provides an integrated approach for prediction of DIVI which aid in target and candidate selection and/or proactive design of development strategies to mitigate these potential risks.

2850 PACAP38 Protects Against Mitoxantrone-Induced Cardiac Injury in Mice

V. Subramanian[2], G. Chuang[2], H. Xia[2], B. Burn[2], J. Bradley[2], J. L. Maderdrut[1] and K. J. Varner[2]. [1]*Medicine, Tulane University, New Orleans, LA and* [2]*Pharmacology, LSU HSC, New Orleans, LA.*

Pituitary adenylate cyclase activating polypeptide 38 (PACAP38) is a member of the secretin/VIP/growth hormone-releasing hormone family of peptides and has many functions, including cell proliferation, regeneration and survival. Mitoxantrone (MXT), a doxorubicin analog, is used to treat multiple sclerosis and some cancers; however, its use is limited by its potential cardiotoxicity. The cardiotoxicity caused by doxorubicin is exacerbated in PACAP -/- mice and reduced by treatment with PACAP38. We tested the hypothesis that PACAP can block MXT-induced cardiotoxicity in mice. Female BALB/c mice were treated 1/week for 4 weeks with saline or MXT (3 mg/kg, ip). The MXT+PACAP group (n=7) received PACAP (10 µg/injection) 1 hr before and 12, 24 and 48 after MXT (time 0). The PACAP group (n=7) received PACAP38 on the same schedule after injection of saline at time 0. Control (n=7) and MXT (n=7) groups were injected with saline on the PACAP38 schedule. Echocardiograms were recorded before and after 2, 3 and 4 weeks of MXT injection. After the 3rd dose of MXT, body weight was significantly reduced and two of the MXT mice died after the 4th dose. PACAP38 significantly attenuated the reduction in body weight; however, the weights of the MXT+PACAP38 mice did not return to control levels. Compared to control mice, MXT-treatment significantly increased left ventricular (LV) diameter and LV volume, coupled with decreased LV posterior wall thickness (LVPW;d). Functionally, fractional shortening (FS) and ejection fraction (EF) were significantly decreased. Treatment with PACAP38 prevented MXT-mediated LV dilation and significantly attenuated the reductions in FS and EF, although FS and EF in MXT-PACAP38 mice did not return to control levels. PACAP38 did not prevent the MXT-induced decrease in LVPW;d. In conclusion, the repeated administration of MXT produces eccentric LV dilation and dysfunction in mice. Moreover, we show that this cardiac toxicity is reduced by the administration of PACAP38. Support P30GM106392.

2851 Functional Integrated Human Cardiac System for Toxicological and Pharmaceutical Studies

C. J. Long[2], C. Oleaga[2], M. Stancescu[2], P. Molnar[2], C. W. McAleer[2], W. McLamb[2], G. Legters[2], J.-M. Prot[1] and J. J. Hickman[2]. [1]*Biomedical Engineering, Cornell University, Ithaca, NY and* [2]*NanoScience Technology Center, University of Central Florida, Orlando, FL.*
Sponsor: E. DuFour.

The pharmaceutical industry invests decades on research for drug development together with billions of dollars to launch them into the market to improve the human condition, but failures still occur in a large percentage of cases. Cardiotoxicity is the number one adverse effect that causes new market releases to be withdrawn. This is indicative of the fact that current regulatory tests fail to accurately predict human cardiotoxicity during the preclinical evaluations. We have developed an *in vitro* human cardiac system that features the two main predictors of cardiac toxicity, conduction velocity and contractile force [1]. Human stem cell derived cardiomyocytes were integrated with a BioMicroelectromechanical system construct for force determination and a microelectrode array for electrical conduction, both in a serum-free medium. Sotalol, norepinephrine and verapamil were used to validate the platform for toxicity and to attempt to differentiate mechanisms of toxicity without the complexity of the whole heart using the two functional readouts. This cardiac system, utilizing a multivariant functional profile, has the ability to reproduce the toxic events that affect the heart as well as address mechanistic aspects, examples of which will be discussed. This novel technology is currently being adapted as a regulatory cardiotoxic platform. [1] Stancescu et al., Biomaterials 60:20-30, 2105.

2852 Comprehensive *In Vitro* Proarrythmic Evaluation of Cardiomyocytes Using a Multi-Electrode Array

M. Renganathan, Y. Zhao, A. Cook, M. Donio and H. Wei. *Eurofins Pharma Discovery Services, St. Charles, MO.*

The current *in vitro* and nonclinical *in vivo* systems are not sufficient for predicting clinical cardiac arrhythmia. Promising drugs are lost during development by using hERG current inhibition as a surrogate marker for QT prolongation. A robust and biologically relevant assessment of potential clinical arrhythmia should include an assessment of multiple human cardiac ionic currents, and human stem cell-derived cardiomyocytes, the two core pillars of Comprehensive *In Vitro* Proarrythmia Assay (CiPA; CSRC/HESI/SPS/FDA meeting, Dec 11, 2014). Eurofins Pharma Discovery Services provides a comprehensive assessment of proarrythmic risk 1) using multiple human cardiac ion channels that are functionally validated in both manual patch clamp and automated electrophysiology platforms, 2) using human Embryonic Stem Cell (hESC) derived cardiomyocytes and induced pluripotent stem cell derived cardiomyocytes (IPS) in the cardiac action potential measurement by conventional patch clamp and 3) extra cellular field potential measurement by Multi-electrode Array (MEA) technology. Here, we describe the characterization of cardiomyocytes from different vendors by measuring the number of beats, beat period, Na+ spike amplitude and field potential duration (FPD) in a multi electrode array (Axion BioSystems). We describe the effect of three sets of compounds that are classified according to the proarrythmic potential; high risk, intermediate risk and low risk.

2853 Short-Term Inhibition of P53 Protects from the Development of Diabetic Cardiomyopathy

J. Gu. *University of Louisville, Louisville, KY.*

Aims/hypothesis: Although previous studies with a focus on the early effect of diabetes have demonstrated that cardiac apoptosis and P53 upregulation at the early stage of diabetes, whether this early apoptotic cell death really plays an important role in the development of diabetic cardiomyopathy (DCM) in the long-term remains to be addressed. Here we examined whether an early, short-period of p53 inhibition and associated apoptotic cell death and senescence can result in a prevention of the late development of DCM and its possible underlying mechanisms. Methods: FVB mice were treated with five daily injections of 50 mg/kg streptozotocin to induce type 1 diabetes. Both hyperglycemic and age-matched control mice were treated with or without PFT-α at 1.1 mg/kg three days a week for 2 months and then these mice were kept without PFT-α treatment until 6 months. At 0.5, 3 and 6 months after diabetes onset, cardiac P53 protein expression was examined by Western blot and immunofluorescence. Apoptosis was measured by TUNEL. Senescence was detected by senescence-associated-β-gal (SA-β-gal) and western blot of P16INK4A. Cardiac function and structure were examined by echocardiography and histopathological evaluation with light microscope. Results: Cardiac P53 phosphorylation (along with TUNEL and senescent positive cells) was significantly increased at 0.5 month after diabetes induction. Further mechanistic studies showed that upregulation of P53 was associated with increases in the Bax/Bcl2 expression ratio, caspase-3 cleavage, AIF expression, CHOP and cleaved caspase-12 but without caspase-8 cleavage change. Moreover, increased p53 phosphorylation was associated with increased SA-β-gal positive staining and P16INK4a expression. In the later disease stage, diabetic mice with early inhibition of P53 were significantly resistant to diabetes-induced cardiac oxidative damage, inflammation, fibrosis, hypertrophy and dysfunction (i.e. DCM). Conclusions/interpretation: These results demonstrate that an early, short-period inhibition of P53 prevents diabetes-induced cardiac senescence and apoptosis, resulting in a prevention of the late development of DCM. Thus P53 may be a therapeutic target for the treatment of DCM.

2854 Assessment of Acrolein-Induced Toxicity Using *In Vitro* Modeling to Evaluate the Role of PARP

K. Harand, J. Coyle, M. Bourgeois, G. Johnson, J. McCluskey and R. Harbison. *Environmental Occupational Health, College of Public Health, Tampa, FL.*

Acrolein is an electrophilic α, β-unsaturated aldehyde. Additionally, acrolein is a metabolite of the antineoplastic alkylating agent cyclophosphamide and is implicated in off-target effects, including bladder hemorrhagic cystitis and cyclophosphamide-induced cardiotoxicity, both of which have led to serious secondary iatrogenic injury during and following chemotherapy. At low concentrations acrolein inhibits cell proliferation without inducing apoptosis, while at high concentrations may result in secondary apoptosis promotion. This investigation assessed the role of the enzyme poly (ADP-ribose) polymerase (PARP) in acrolein induced toxicity using the established toxicological H9c2 (2-1) Cardiomyoblast *in vitro* model. H9c2 (2-1) cells were plated in 24-well plates at 75,000 cells per well three days prior to testing, followed by acrolein dosing at concentrations between 10 µM and 1000µM for either 30 or 55 minutes. PARP activity was quantitatively measured in total cell lysates using a biotin-avidin-conjugated horseradish peroxidase-TMB reporter system in a 96-well microplate. The lowest effective dose of toxicity at 30 minute dosing was found at 25 µM (PARP Activity 1.65-fold control) which returned to baseline at 100 µM; concentrations at or above 250 µM resulted in significant PARP activity reductions (≤ 0.46-fold control). This evidence suggests that acrolein induced cardiotoxicity may be modulated through PARP inhibition.

2855 Inhalation Exposure to Traffic-Derived Particulates Diminishes Left Ventricular Performance and Alters Myocardial Protein Expression and Signal Transduction Consistent with Sympathetic Mediation in Rats

A. P. Carll[4,5], T. D. da Silva[2,5], B. T. Lima[1,5], M. S. Filho[1,5], R. D. Raimundo[2,5], S. M. Crespo[1,5], D. H. Zati[1,5], V. Chandrasekhar[3], A. L. R. Vitor[2,5], G. Pyrgiotakis[5], E. A. Diaz[5], M. I. Kontaridis[3] and J. J. Godleski[5]. [1]*Faculdade de Medicinade Universidade de São Paulo, São Paulo, Brazil;* [2]*Universidad Federal de São Paulo, São Paulo, Brazil;* [3]*Cardiovascular Division, Beth Israel Deaconess Medical Center, Boston, MA;* [4]*Diabetes and Obesity Center, University of Louisville, Louisville, KY and* [5]*Environmental Health, Harvard T. H. Chan School of Public Health, Boston, MA.*

Vehicular traffic is a major source of fine particulate matter (PM2.5), a ubiquitous air pollutant associated with increased cardiovascular morbidity and mortality. Inhaled traffic-derived PM2.5 (TPM) may exert cardiotoxicity by increasing sympathetic neural influence over cardiovascular function, but this mechanism remains unconfirmed and the dose-effect relationship poorly understood. We chronically implanted rats (n=7/group) with telemeters with left ventricular (LV) pressure catheters and electrodes to test if a 16-day exposure to TPM (187 µg/m3, 5 h/day, 4 d/wk, 4 wk) would alter LV performance, heart rate (HR), heart rate variability (HRV), and electrocardiogram (ECG) during exposure and during acute stress tests, and alter cardiac signal transduction, relative to filtered air-exposed rats. During psychosocial stress after 4 days of exposure, TPM augmented inotropic, lusitropic, hemodynamic, and HR responses, suggesting enhanced sympathetic regulation. From day 11 of exposure onward, TPM decreased contractility and lusitropy relative to filtered air. After the 16-day exposure, TPM caused greater arrhythmias and HRV indications of sympathetic dominance during social stress relative to air exposure. TPM altered myocardial signaling and protein expression consistent with sympathetic dominance and remodeling in separate non-telemetered rats (n=6/group). Our findings suggest repeat exposure to traffic-derived PM at near-ambient levels decreases LV performance toward heart failure, exacerbates arrhythmic and sympathetic responses to stress, and activates signal transduction pathways associated with sympathetic dominance and myocardial remodeling. PM exposure may confer cardiac toxicity, promote heart failure, and potentiate naturally triggered arrhythmia via sympathetic pathways.

2856 Rats Treated with Doxorubicin Had Reduced Cardiac Function Demonstrated by MRI and Echocardiography Measurements

K. Henderson[1], A. Bratasz[2], K. Powell[2], J. Ross[1], R. B. Borders[1], B. M. Roche[1] and T. Vinci[1]. [1]*Battelle, Columbus, OH and* [2]*Heart and Lung Institute, The Ohio State University, Columbus, OH.*

Doxorubicin (dox) is a widely used oncology drug with known cardiotoxicity that can manifest years after treatment. The purpose of this study was to simulate a clinical treatment regimen in Sprague Dawley rats to determine if impairment of cardiac function is detectable utilizing cardiac magnetic resonance imaging (MRI) to monitor cardiac output (CO), stroke volume (SV), ejection fraction (EF), end systolic volume (ESV), end diastolic volume (EDV), and heart rate (HR). In addition, echocardiography was performed as a comparison to MRI assessment. The treatment group (n=5) was administered dox as an IV dose of 2 mg/kg once/week for 4 weeks for a cumulative dose of 8 mg/kg. A control group (n=3) was administered saline in the same manner. Animals were anesthetized with isoflurane and imaged using MRI once predose and then one week post doses on Day 30. Echocardiography measurements were made predose, Day 14, and Day 28. Differences between predose and postdose measurements for cardiac function were compared between the control and treatment groups. Cardiac function in the dox group was significantly lower by day 30 compared to the saline group. CO in the saline group increased over 30 days by 34.9±10.9% versus 14.8±10.9% for the dox group (~58% lower). SV increased by 33.1±13.3% versus 20.1±10.3% (appox. 40% lower) for the saline versus the dox group, respectively. Mean EDV was ~62% less and average ESV was ~79% less in the dox group compared to the saline group. Heart rates were similar for both groups. Echocardiography revealed a similar trend between the treatment groups with the actual measured values being lower than MRI measurements: echocardiography measurements in the dox group for EDV and ESV were 0.307±.042 mL and 0.076±0.020 mL, respectively versus 0.594±0.021 mL and 0.168±0.002 mL respectively as measured by MRI. Cardiac dysfunction was apparent following a cumulative dose of 8 mg/kg of dox in Sprague Dawley rats by measurements using MRI and echocardiography over 30 days.

2857 Drug-Induced Contraction Force Analysis of Cardiomyocyte - Cardiac Fibroblast Co-Cultures Using the "CellDrum" Technology

M. Gossmann[1], T. Palm[1], E. Kolossov[1], G. Luerman[2], A. Duenbostell[1], M. Torvinen[3], G. Artmann[3] and H. Bohlen[1]. [1]*Axiogenesis AG, Cologne, Germany;* [2]*Axiogenesis Inc, Plymouth Meeting, PA and* [3]*Fachochschule Aachen, Jülich, Germany.* Sponsor: E. Clarke.

To assess drug-induced cardiotoxicity, new cell models and technologies are currently in focus to reach new standards in compound screening during early drug development. Human iPSC-derived cardiomyocytes represent a homogenous and reproducible cell model that allows analysis of potential drug candidates on different cellular functions such as cell metabolism and force of contraction. To analyze drug-induced changes of mechanical properties such as contractility, we employed a novel technology termed "Cell Drum" that measures and calculates cellular tension from the deflection of a myocyte-layered membrane and the pressure beneath. The Cell Drum was combined with a co-culture of human iPSC-derived cardiomyocytes (Cor.4U) and cardiac fibroblasts (Fibro.Cor.4U) to further push *in vitro* cell models to a more physiologically relevant level. Ten different compounds with known cardioactivity were quantified on mechanical properties of the co-cultures using the Cell Drum technology, including ion channel modulators, sensitizers and adrenergic agents. Cell metabolism changes of co-cultures upon drug induction was further assessed with the Seahorse XF 96 analyzer. Co-culture of cardiomyocytes and cardiac fibroblasts produced optimal force in the Cell Drum (at 25-50% fibroblasts) and better respiratory performance using the Seahorse technology in comparison to normal cultures or co-cultures with lower cardiac fibroblast fraction. These new technologies, combined with human, physiologically relevant cell models better reflecting *in vivo* conditions, provide a significant advancement for cardiac safety/toxicology analysis.

2858 Cardiovascular Toxicity of Nanoparticles to Zebrafish

J. Gao, M. S. Sepúlveda and C. Mahapatra. *Forestry and Natural Resources, Purdue University, West Lafayette, IN.*

Nanotechnology manipulates matter at the nanoscale (1-100 nm) is rapidly moving into many aspects of human life. Engineered nanomaterials (ENMs) can enter into the environment through air, water and food chains, which can result in exposure to humans and other organisms leading to potential adverse health effects. However, specific effects on the cardiovascular system by ENMs have been mostly overlooked. Our study was focusing on the effects of nanoparticles (NPs) on cardiovascular development using zebrafish (*Danio rerio*) embryos. Our studies demonstrated that polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNPs, 1- 10 ppm) (60 nm) caused angiogenesis delay during early life stages (24 and 48 hours post-fertilization, hpf) and abnormal vascular development at later life stages (72 and 96 hpf). In addition, PVP-AgNPs also caused heart development delay resulting in bradycardia, decreased respiration and overall growth delays. Finally, we observed an induction in the expression of several genes (vegf, vegfr, pi3k, pkc, plc, and bcl2) related with the vascular endothelial growth factor (VEGF) pathway. Interestingly, VEGF protein level was not increased, suggesting disruption of translational control mechanisms. In addition, the negative effects caused by PVP-AgNPs cannot be attributed to silver ions (Ag+) since PVP-AgNPs during exposure experiment were stable, and the concentrations of Ag+ in all PVP-AgNPs treated groups were below 1 µg/L. In conclusion, our results show that PVP-AgNPs are anti-angiogenic, and vasculo-and cardio-toxic and that these effects are related with the VEGF pathway.

2859 Inhaled Toxicity of Titanium Dioxide Nanoparticles Deteriorate ApoE-/- Mice Atherosclerosis

B.-J. Lee[1], M. Kang[1], D.-H. Yoo[1] and K. Lee[1,2]. [1]*Department of Inhalation Toxicity, KIT, Jeonbuk, Korea, Republic of and* [2]*Human and Environment Toxicology, University of Science and Technology, Daejeon, Korea, Republic of.* Sponsor: K. Lee.

Titanium dioxide (TiO2) is used in human life widely such as the occupational workplace, consumer products, cosmetics and food material. In spite though, TiO2 has been only assessed toxicity entering the body via pulmonary exposure research extra pulmonary tissues including blood, liver, kidney, and brain many decade ago. However, researches on the cardiovascular adverse effects of nanometer-sized TiO2 are lacking. Here we studied an atherosclerosis the relationship between nanometer-sized TiO2 and atherosclerosis with Apolipoprotein E knockout

(ApoE-/-) mice model. We were exposure the nanometer-sized TiO2 of 1.2 mg/m3 of low dose group and 12 mg/m3 of high dose group to ApoE-/- mouse by nose only inhalation chamber for six hours per a day, four weeks, while the control group exposure on filtered fresh air with a same systems. We observed survival rates, food consumption, bio-makers of atherosclerosis (adiponectin, fibrinogen, 8-isoprostane, oxidized low-density lipoprotein (OX-LDL)) with serum, and histopathology to determine the atherosclerosis and cardiac disorder. The results were shown that TiO2 inhaled mice were not observed the significant differences survival rate and food consumption compared with control group. However, there was difference observed TiO2 exposed groups in terms of total cholesterol, phospholipid compared between the normal reference animal. Furthermore, total bilirubin in serum chemistry was increased both TiO2 exposed groups; whereas triglyceride was detected decreased TiO2 exposed groups as does dependent manners. In addition, histopathology study revealed that an increased size of the atherosclerotic plaques and mineralization were observed at aorta and femoral veins TiO2 inhaled groups. Our studies showed that nano-size particle of TiO2 are induced systemic atherosclerosis in ApoE-/- mouse model. These results therefore provide strong evidence for exposed TiO2 deteriorates atherosclerosis in the subtle systemic effects of early steps of cardiac disorders.

PS 2860 *In Vitro* Characterization of the Cutaneous Immunotoxicity of Reactive and Disperse Textile Dyes

D. M. Leme[3], A. Sehr[1], T. Grummt[1], T. Jacomasso[3], S. M. Winnischofer[3], F. G. Arnoldi[3], G. A. Oliveira[2], M. C. Valadares[2], F. B. Potrich[3], C. C. Oliveira[3], D. F. Buchi[3], E. S. Trindade[3] and D. P. Oliveira[3]. *[1]Umweltbundesamt, Bad Elster, Germany; [2]Universidade Federal de Goiás (UFG), Goiânia, Brazil and [3]Universidade Federal do Paraná (UFPR), Curitiba, Brazil.*

The increase number of contact dermatitis cases associated with textile dyes has led to the attention for identifying dyes with the potential to cause cutaneous inflammation aimed to protect consumers. However, the accuracy in identifying immunotoxic chemicals has faced the challenge of not use animal testing for toxicological purposes. Several *in vitro* methods, comprising the analysis of different inflammatory mediators, have been proposed as alternatives of animal-based assays. Three reactive [Reactive Green 19 (RG19), Reactive Blue 2 (RB2), Reactive Black 5 (RB5)] and one disperse [Disperse Red 1 (DR1)] textile dyes, being two of them already classified as skin sensitizer (RB5 and DR1), were assessed regarding their potential to induce cutaneous inflammatory reactions using *in vitro* keratinocytes-based methods. HaCaT cells (immortalized human keratinocytes) exposed to non-cytotoxic concentrations of the tested dyes (48 h) and their respective solvent controls (PBS, DMSO) were assessed using biomarkers of extracellular matrix remodeling (MMP-2, MMP-9, TIMP-1, TIMP-2, RECK - RT-qPCR, gelatin-zymography) and inflammatory cytokine profiles [RT-qPCR, BD Cytometric Bead Array (CBA) Human Inflammatory Cytokines kit]. DR1 was the only dye able to trigger an immune response (expression and activity of MMP-2 and expression and release of particular cytokines IL-8, IL-6, IL-12), which may be considered as skin immunotoxicant. The reactive dyes, including RB5 that was previous reported as skin sensitizer according to literature, were not able to induce inflammatory reactions, under the conditions tested. Even that, the reactive dyes studied may pose a risk to human keratinocytes by the induction of effects related to modulation of MMP-9, which is involved in a variety of pathophysiological conditions, such as carcinogenesis. Thus, all these dyes should be used with caution to avoid undesirable effects to consumers when dermally exposed. Financial Support: FAPESP 2010/14941-3.

PS 2861 Development of an *In Vitro* Method to Estimate the Sensitization Induction Level Using Reconstituted Human Epidermis

E. Corsini, V. Galbiati, A. Papale, E. Kummer, M. Marinovich and C. L. Galli. *DiSFeB, Università degli Studi di Milano, Milan, Italy.*

Keratinocytes are implicated in the maturation and migration of dendritic cells by releasing several factors, including interleukin-18 (IL-18). We previously demonstrated in NCTC2544 cells that a selective induction of IL-18 by contact allergens (Corsini et al. 2009). More recently, the possibility to combine the NCTC2544-IL 18 assay and the reconstructed human epidermis (RHE)-based assay for irritation/corrosion to identify and classify according to their potency contact allergens has been described (Gibbs et al., 2013). The purpose of this study was to extend the list of tested chemicals, and to define the applicability domain of the assay. As RHE model, Epiderm™ (MatTek) was used throughout the study. 15 chemicals (10 contact allergens and 5 irritants) were tested in two independent experiments. DNCB (3 mg/ml) in AOO (4:1) was used a positive control. For benzoquinone, benzylsalicylate and benzylcinnamate, an interference with the readouts (MTT assay and/or IL-18 ELISA) was observed, which may limit the applicability domain. The IL-18 stimulation index (SI) values obtained for the positive control DNCB ranged from 6.2 to 24.8, indicating some variability between different RHE batches. All chemical tested lacking the ability to induce skin sensitization in humans failed to induce IL-18 release. An IL-18 SI 2.5-3.0 is proposed for the hazard identification of contact allergens, while for potency assessment using reference contact allergens (i.e. DNCB, isoeugenol, eugenol and benzocaine), standard curves were created by plotting *in vivo* LLNA EC3 vs *in vitro* EC50 or IL-18 SI2 to extrapolate *in vivo* LLNA EC3 value using *in vitro* values, which could be useful in risk assessment. Acknowledgement. This research was supported by the Alternatives Research & Development Foundation's 2014 Alternatives Research Grant Program. References: Corsini E et al. Toxicol *In Vitro*. 23, 789-96, 2009. Gibbs S et al. Toxicol Appl Pharmacol. 272, 529-41, 2013.

PS 2862 The Immunomodulatory Potential of microRNA 210 and Regulatory T Cells in a Murine Model of Chemical Sensitization

C. M. Long[1,2], N. Marshall[1], E. Lukomska[1], H. Shane[1], P. Siegel[1], B. J. Meade[1] and S. Anderson[1]. *[1]Allergy and Clinical Immunology Branch, CDC-NIOSH-HELD, Morgantown, WV and [2]Immunology and Microbial Pathogenesis Graduate Program, West Virginia University, Morgantown, WV.*

Toluene 2,4-diisocyanate (TDI) is a low molecular weight chemical sensitizer and a major cause of occupational asthma. The roles of immunomodulatory elements such as microRNAs, which alter gene expression, and regulatory T cells (Tregs), which suppress T cell-mediated responses, in TDI sensitization are unknown. We have previously shown that miR-210 expression in the draining lymph node (dLN) and ear is augmented during TDI sensitization. In order to further investigate the functional role of miR-210 and Tregs during TDI sensitization, BALBc mice were dermally exposed to a single, sensitizing dose of TDI (0.5-4% v/v) and evaluated for immunological endpoints. Alterations in expression of putative miR-210 transcription factor targets were observed in the dLN during TDI sensitization. These transcription factors are directly involved in Treg expansion and function; therefore, miR-210 may potentially negatively target Treg differentiation and function during TDI sensitization. dLN Tregs were phenotyped during TDI sensitization and a heterogeneous population was observed. To examine Treg functionality, a carboxyfluorescein succinimidyl ester-based Treg suppression assay was conducted. Tregs isolated from the dLNs of TDI sensitized mice were more suppressive than their control counterparts. The Treg population was then depleted prior to TDI sensitization by *in vivo* administration of a CD25-targeting antibody. The sensitization response was intensified following Treg depletion, as evidenced by amplified total dLN cellularity, Th2 expansion, and IL-4 mRNA production. These novel findings indicate that Tregs may play an important role in modulating allergic responses during TDI sensitization and that miR-210 may have an inhibitory role in this process. These data are necessary to fully understand the immunologic mechanisms of chemical-induced allergic disease and may aid in the development of preventative and therapeutic strategies.

PS 2863 THP-1 in Coculture with HaCaT Keratinocytes (COCAT) for an Advanced *In Vitro* Prediction of Sensitization Potential and Potency of Chemicals

J. Hennen and B. Blömeke. *Department of Environmental Toxicology, University Trier, Trier, Germany.*

Many ingredients of daily life consumer products can act as contact allergens, therefore knowledge regarding their sensitization potential is crucial. Here, the potential of a chemical to interact with proteins, activate keratinocytes and dendritic cells (DC) and finally provoke T cell proliferation and differentiation are considered as hallmarks within the adverse outcome pathway of skin sensitization. Several alternative tests addressing single steps have been established for hazard prediction, but estimation of sensitization potency is still challenging. To combine factors such as keratinocyte xenobiotic metabolism and skin inflammation with DC activation, we exposed THP-1 cells in coculture with HaCaT keratinocytes to sensitizers (haptens, pro- and prehaptens). The presence of HaCaT cells enhanced maximal upregulation of cell surface molecules CD86 and/or CD54 on THP-1 cells. Using Dmean fluorescence intensity (ΔMFI) of CD86 ≥ 10 and/or ΔMFI of CD54 ≥ 50 as thresholds for positivity, we found an overall accuracy of 96% and a significant correlation of the chemicals' concentrations needed to exceed these thresholds, namely ECD10 (CD86) and ECΔ50 (CD54), with human and animal data on sensitization potency. In this study, we transferred and optimised the protocol of our cocultured activation test (COCAT) for its application in a 96 well plate format. Analyzing 15 sensitizers (5 haptens, 6 prohaptens,

4 prehaptens) and 5 non-sensitizers using the standardized protocol in 3 repetitions yielded a positive result (at least 2/3 runs positive) for 14/15 sensitizers, while the 5 non-sensitizers gave negative results, yielding a high capacity (95% accuracy) for hazard prediction. Furthermore, we observed a significant correlation of results obtained in the COCAT with *in vivo* data on sensitization potency. These data indicate that the COCAT, integrating keratinocyte responses, metabolism and DC activation, has the potential to fill the gap regarding the prediction of sensitization potency.

2864 Evaluation of 4-methylcyclohexanemethanol in a Combined Irritation and Local Lymph Node Assay in BALB/c Mice

V. J. Johnson[1], S. S. Auerbach[2], M. I. Luster[1], S. Waidyanatha[2], S. A. Masten[2], M. S. Wolfe[2], F. G. Burleson[1], G. R. Burleson[1] and D. R. Germolec[2]. [1]*Burleson Research Technologies, Inc., Morrisville, NC and* [2]*National Toxicology Program, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC.*

4-Methylcyclohexanemethanol (MCHM) is a flotation reagent used in fine coal beneficiation. On January 9, 2014 crude MCHM (~88.5% MCHM) was inadvertently released into the Elk River in Charleston, WV resulting in temporary contamination of 15% of the state's tap water and causing significant dermal exposure. These studies were undertaken to determine whether crude MCHM or pure MCHM has the potential to produce dermal irritation and/or sensitization. Female BALB/c mice were treated daily for 3 consecutive days by direct epicutaneous application of 25 µL of various concentrations of crude or pure MCHM to the dorsum of each ear. A mouse ear swelling test, used to determine irritancy potential, was conducted in combination with the Local Lymph Node Assay (LLNA) to determine dermal sensitizing potential. Dermal exposure to pure MCHM caused irritation of the skin at the application site at concentrations above 20% and overt toxicity at the 100% concentration, but did not induce sensitization. Mice treated with ≥75% crude MCHM also showed evidence of dermal irritation, although weaker when compared to pure MCHM. Overt toxicity was also observed in mice treated with 100% crude MCHM, although the severity was less than pure MCHM. Dermal application of crude MCHM resulted in increased lymphocyte proliferation in the draining lymph node at concentrations ≥5%. The Stimulation Index (SI), a measure of sensitization, was significantly increased in mice treated with ≥20% crude MCHM, relative to the vehicle control group. The SI was above 3, the threshold for positive sensitization potential, following dermal application of ≥40%. These results indicate that crude MCHM has the potential to cause dermal sensitization at exposure concentrations that are non-irritating. This work was supported by NIH contract HHSN273201400017C

2865 Integrated Testing Strategy for Skin Sensitization Assessment

A. Del Bufalo[2], C. Piroird[2], H. Nocairi[2], J. Eilstein[2], A. Detroyer[2], C. Gomes[2], T. Pauloin[1] and S. Teissier[2]. [1]*L'Oreal, Asnières, France and* [2]*L'Oreal, Aulnay sous Bois, France.* Sponsor: E. DuFour.

Skin sensitization is a delayed type allergy consisting of a cellular immune reaction to electrophilic small molecular weight chemicals. Since March 2013, the 7th Amendment of the Cosmetics Directive prohibits in Europe the marketing of cosmetic products containing ingredients which were tested on animal-based assays and prompted the implementation of Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitization. While there is a common understanding of the Adverse Outcome Pathways (AOP's) leading to skin sensitization, as well as a wide appropriation of a core battery of assays addressing these AOP key events, the ways of integrating such data to allow risk assessment of new ingredients is still a challenge. For that purpose, we generated a complete training set of data using 8 parameters from *in silico* predictions models (TIMES,Toxtree), from DPRA, U-SENSTM, KeratinoSens and PGE-2 assays as well as two physicochemical parameters (volatility and pH) on 165 substances covering the diversity of cosmetic ingredients and having an LLNA-based S/NS classification. We submitted it to statistical analysis: from the large number of supervised classification models proposed in the literature, we chose five different methods: Boosting, Naïve Bayes, SupportVectorMachine (SVM), Sparse PLS-DA and Expert Scoring. These methods have strong differences, but they all produce posterior probability of belonging to the group of interest ("sensitizer"). We combined them by the stacking methodology of Wolpert and Breiman, in order to obtain a specific "stacking" meta-model. Results from this two classes (Sensitizer/Non Sensitizer) prediction meta-model obtained on a validation data set show predictive performances of 93 % concordance, 96% sensitivity and 90 % specificity. Based on this experience on cosmetic case studies we will design tiered testing strategies to insure consumer safety while fitting to industrial needs.

2866 Irritancy and Allergic Responses Induced by Topical Application of Didecyldimethylammonium Chloride

S. E. Anderson, H. Shane, C. Long, E. Lukomska, B. J. Meade and N. B. Marshall. *CDC NIOSH, Morgantown, WV.*

Didecyldimethylammonium chloride (DDAC) is a dialkyl-quaternary ammonium compound that is used in numerous products for its bactericidal, virucidal and fungicidal properties. There have been reports of immediate and delayed hypersensitivity reactions following occupational exposure to this chemical, however the sensitization potential of DDAC has not been thoroughly investigated. The purpose of these studies was to evaluate the irritancy and sensitization potential of DDAC following dermal exposure. DDAC induced significant irritancy, evaluated by ear swelling, when female Balb/c mice were treated with concentrations 0.5% and higher. Initial evaluation of the sensitization potential was conducted using the local lymph node assay (LLNA) at concentrations ranging from 0.0625% to 1%. A dose responsive increase in lymphocyte proliferation was observed reaching statistical significance at 0.25% (non-irritating) with a calculated EC3 value of 0.17%. Dermal exposure to DDAC did not induce production of IgE as evaluated by phenotypic analysis of draining lymph node cells (IgE+B220+) and measurement of total serum IgE levels. Additional phenotypic analysis revealed significant and dose-responsive increases in the absolute number of B-cells, CD4+ T-cells, CD8+ T-cells and dendritic cells, along with significant increases in the percent of B-cells (0.25% and 1%) and decreases in CD4+ (0.25-1%) and CD8+ (1%) T-cells at day 10 following 4 days of dermal exposure. There was also a significant and dose-responsive increase in the number of activated CD4+, CD8+, B-cells and dendritic cells following exposure to all concentrations of DDAC. These results demonstrate the potential for development of irritation and hypersensitivity responses to DDAC following dermal exposure and raise concern about occupational and consumer use of this chemical.

2867 Libby Amphibole-Induced Mesothelial Cell Autoantibodies Bind to Surface Plasminogen and Alter Collagen Matrix Remodeling

R. Hanson[2], C. Evilia[2], L. Woods[2], J. Gilmer[2] and J. C. Pfau[2]. [1]*Biological Sciences, Idaho State University, Pocatello, ID and* [2]*Chemistry, Idaho State University, Pocatello, ID.*

Lamellar Pleural Thickening is a fibrotic disease induced by exposure to Libby Amphibole (LA) asbestos that causes widespread scarring around the lung, resulting in progressive loss of pulmonary function. Investigating the effects of autoantibodies to mesothelial cells (MCAA) present in the study populations has been a major part of the effort to understand the mechanism of pathogenesis. It has been shown *in vitro* that mesothelial cells (Met5a) exposed to MCAA increase collagen deposition into the extracellular matrix (ECM). In this study, we sought to further elucidate why the presence of MCAA would result in increased collagen deposition by identifying the protein targets bound by MCAA on the cellular surface using biotinylation to label and isolate surface proteins. Protein targets were selected by immunoprecipitation and MCAA binding via ELISA. The fractions that demonstrated binding by MCAA were then analyzed by tandem Mass Spectrometry and MASCOT analysis. We identified annexin A5, cytoskeletal keratin 18, and plasminogen as possible candidate targets that could affect the regulation of the ECM. The most promising result from the MASCOT analysis, plasminogen, was tested for MCAA binding using purified human plasminogen in an ELISA. We report that serum containing MCAA bound at an optical density (OD) 3 times greater than that of controls, and LA-exposed subjects had a high frequency of positive tests for anti-plasminogen autoantibodies. This work implicates the involvement of the plasminogen/plasmin system in the mechanism of excess collagen deposition in Met5a cells exposed to MCAA. Elucidating this mechanism could contribute to the understanding of LPT.

2868 Libby Amphibole-induced Mesothelial Cell Autoantibodies Promote Collagen Deposition in Mice

J. Gilmer[1], K. Serve[2], R. Hanson[1] and J. C. Pfau[1]. [1]*Biological Sciences, Idaho State University, Pocatello, ID and* [2]*Biology, Mars Hill University, Mars Hill, NC.*

Libby Amphibole (LA) asbestos has been shown to cause a unique and progressive lamellar pleural fibrosis (LPF) that is associated with severe declines in pulmonary function. This disease is also associated with the production of anti-mesothelial cell autoantibodies (MCAA), which can induce collagen production from cultured human mesothelial cells.

We hypothesized that the progressive nature of this LPF can be at least partially attributed to an autoimmune process. This study sought to demonstrate that LA induces MCAA that can trigger collagen deposition *in vivo*. C57BL/6 mice were exposed to LA for 7 months, and serum was collected and tested for MCAA by cell-based ELISA on primary mouse mesothelial cells. When treated *in vitro* with serum from mice exposed to LA, mouse mesothelial cells significantly upregulated collagen matrix production. This effect was lost when the serum was cleared of IgG using Protein G beads, demonstrating that the effect was mediated by IgG autoantibodies. Using the peritoneal cavity as a surrogate for the pleural cavity, groups of naïve (non-asbestos-exposed) mice were injected intraperitoneally with a) serum from saline treated mice, b) 1 dose of serum from LA-exposed mice, c) 2 doses of serum from LA-exposed mice, and d) 2 doses of serum from LA-exposed mice cleared of IgG. After 1 month, mice were euthanized and peritoneal walls were harvested. Analysis of collagen density using 2-photon confocal microscopy (SHG analysis) and a hydroxyproline assay demonstrated significant increases in collagen by serum from LA-exposed mice, but not cleared serum. Increased soluble collagen in the peritoneal wash was seen in mice exposed to the MCAA-containing serum for only 1 week. These data strongly support the hypothesis that MCAA in LA-exposed mice are able to induce fibrosis *in vivo*, demonstrating that an autoimmune component may be contributing to the progressive pleural fibrosis seen in LA-exposed patients.

PS 2869 Inhibitory Effect of Subchronic Low Dose 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Experimental Autoimmune Encephalomyelitis

B. Kaplan, J. Stokes and E.-J. Yang. *Center for Environmental Health Sciences, Department of Basic Sciences, Mississippi State University, Mississippi State, MS.*

Multiple sclerosis (MS) is an autoimmune neurodegenerative disease, characterized by demyelination in the central nervous system leading to limb paralysis. Experimental autoimmune encephalomyelitis (EAE), induced by immunization with a self peptide, myelin oligodendrocyte glycoprotein (MOG), has been established to evaluate the pathogenic mechanisms of MS in mice. Many studies have demonstrated that the infiltration of IFN-γ- and IL-17-producing CD4+ T cells (TH1 and TH17 cells, respectively) and downregulation of regulatory T cell (Treg) function are the main contributors to EAE development. The environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been shown to suppress EAE pathogenesis by suppressing CD4+ T and inducing Treg cells in an AHR-dependent manner. However, many previous studies have examined the TCDD effect following an acute high dose (50 µg/kg once). Therefore, the goal of this work was to investigate the immunomodulation of antigen-specific immune responses with subchronic low dose TCDD (1 µg/kg/day for 12 days) in EAE. MOG-specific responses in splenocytes were evaluated at early (3 days post immunization) and late (> 18 days) stages of EAE. The results indicate that subchronic low dose TCDD attenuated EAE clinical scores. The mechanism by which TCDD attenuated EAE involved suppression of IFN-γ in the serum, suppression of MOG-specific CD4+IFN-γ+ responses in the spleen, suppression of IFN-γ by splenocytes in culture medium, and induction of splenic CD4+CD25+FoxP3+ Tregs. Interestingly, MOG-specific CD4+IL-17+ responses in the spleen were increased, and serum IL-17 was slightly elevated, but IL-17 released by splenocytes in culture medium was significantly inhibited, suggesting subchronic low dose TCDD exposure produces different effects on IL-17 versus IFN-γ responses.

PS 2870 Autoimmune Potential of Perchloroethylene: Role of Lipid-derived Reactive Aldehydes

G. Wang, J. Wang, G. Ansari and M. F. Khan. *Pathology, University of Texas Medical Branch, Galveston, TX.*

Perchloroethylene (PCE) exposure has been implicated in the pathogenesis of autoimmune diseases (ADs). However, experimental studies exploring the potential of PCE in inducing autoimmunity are lacking. Therefore, we examined the role of PCE in the induction and/or exacerbation of autoimmune response by performing time- and dose-response studies. Six-week old female MRL+/+ mice, in groups of 6 each, were treated with PCE (0.25, 0.5 or 1.0 mg/ml) via drinking water for 12, 18 and 24 weeks and markers of lipid peroxidation and autoimmunity were determined. Significant increases in anti-malondialdehyde (MDA)- and 4-hydroxynonenal (HNE)-protein adduct antibodies were found in the sera of PCE-treated mice with a time-dependent response. The increases in anti-MDA-/HNE-antibodies were associated with significant increases in serum ANA and anti-dsDNA antibodies at 18 weeks and, to a greater extent at 24 weeks, suggesting that PCE exposure exacerbated autoimmunity in our animal model. The splenocytes from mice treated with PCE for 18 and 24 weeks showed higher Th17 cell proliferation following 72 h stimulation with MDA-mouse serum albumin adducts, suggesting that MDA-modified proteins may act as an immunologic trigger by activating Th17 cells leading to an autoimmune response. PCE treatment also led to hair loss in the chin area, back and belly of mice along with dermatitis lesions around 19 weeks. Our studies provide the experimental evidence that PCE induces/exacerbates an autoimmune response and lipid-derived reactive aldehydes contribute to this response. Supported by NIH ES023106.

PS 2871 Changes of Dopamine and Serotonin Levels in Plasma and Brain of 79 Weeks old Normal and Diet Induced Obese Experimental Autoimmune Encephalomyelitis Mice

M. Hasan[1,3], J.-E. Seo[1,3], M.-J. Kang[1,2], B.-H. Jung[1,2] and O.-S. Kwon[1,3]. [1]*Department of Biological Chemistry, Korea University of Science and Technology, Daejeon, Korea, Republic of;* [2]*Molecular Recognition Research Center, Korea Institute of Science and Technology, Seoul, Korea, Republic of and* [3]*Toxicology Lab., Doping Control Center, Korea Institute of Science and Technology, Seoul, Korea, Republic of.*

Obesity and aging are known as contributing factors in disease severity of experimental autoimmune encephalomyelitis (EAE) mice. Monoamine neurotransmitter dopamine (DA) and serotonin (5-HT) play important physiological roles in brain and periphery. The aim of current study is to investigate the changes of DA and 5-HT levels in plasma and brain during disease progression of 79 weeks old normal and obese EAE mice. Male C57BL/6J mice (four weeks old) were kept either in a normal diet (ND) or high-fat diet (HFD) for 75 weeks. EAE was induced in 79 weeks old ND and HFD mice by immunization with myelin oligodendrocytes glycoprotein (35-55). Although, the day of onset, disease incidence have no significant differences between ND- and HFD-EAE mice, from day 30, HFD-EAE mice had shown progressive clinical scores than ND-EAE mice. MMP-9 and NADPH oxidase activities were measured in plasma, spinal cord, and adipose tissue confirmed their increased activities in EAE mice than control mice. The levels of DA, 5-HT and their metabolites in plasma and brain were measured through high-performance liquid chromatography. HFD-mice had shown significantly lower DA levels in plasma than ND-mice. Moreover, DA level was significantly increased time-dependently in ND-EAE mice than ND-CON and HFD-EAE mice. However, no significant differences in DA level were observed between HFD-CON and HFD-EAE. Likewise DA, the 5-HT level was significantly higher in ND-EAE than ND-CON at day 20 and 30. However, the levels of DA and 5-HT in the brain was below detection limit as aging lowers monoamine levels in the brain. Lower lymphocytes count in both ND- and HFD-EAE mice compared to control mice, and lower WBC count in only ND-EAE mice than control mice were observed. An altered pro- and anti-inflammatory cytokine profiles were also obtained in ND- and HFD-EAE mice compared with control mice. Therefore, the increased DA and 5-HT might act on D1 and 5-HT1A receptors respectively and promote polarization of naïve CD4+ T cells towards Th17 cells and/or T cells proliferation.

PS 2872 Alterations in Lipid and Amino Acid Metabolism on Experimental Autoimmune Encephalomyelitis with Aging Effect Based on Metabolomic and Genomic Approaches

J.-E. Seo[1,4], S. H. Lee[2], M. Hasan[1,4], M.-J. Kang[1,4], B. H. Jung[1,4] and O.-S. Kwon[1,4]. [1]*Department of Biological Chemistry, Korea University of Science and Technology, Daejeon, Korea, Republic of;* [2]*Department of Medical Records and Health Information Management, College of Nursing and Health, Kongju National University, Seoul, Korea, Republic of;* [3]*Molecular Recognition Research Center, Korea Institute of Science and Technology, Seoul, Korea, Republic of and* [4]*Toxicology Laboratory, Doping Control Center, Korea Institute of Science and Technology, Seoul, Korea, Republic of.*

Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model of multiple sclerosis which is a demyelinating disease of the CNS. We have studied on the influence of aging in EAE development and defined that EAE severity is significantly higher in aged group than young group. To elucidate which biomarkers contribute to EAE disease progression with aging, metabolomic profiling of plasma and genomic profiling of brain were performed from 6 weeks and 15 months female C57BL/6J mice. In the metabolite analysis, linoleic acid metabolism was activated and the level of most unsaturated fatty acids and essential amino acids such as histidine, lysine, leucine, and tryptophan were lower in EAE compared to control. In particular, the level of sphingolipid metabolite in aged EAE was clearly higher than aged

control group, while the changes in young group were not. Metabolic pathway analysis showed that unsaturated fatty acids and tryptophan metabolism were significantly associated to EAE development regardless of age, but sphingolipid and tyrosine metabolism had higher p-values in aged EAE compared to young EAE. cDNA microarray analysis also showed that genes participating in sphingolipid metabolism (alkaline ceramidase 2), tyrosine metabolism (dopa decarboxylase) and linoleic acid metabolism (phospholipase A2) were significantly up-regulated in aged EAE compared to young EAE. As a result, the changes in lipid and amino acid metabolism contribute to EAE development, most of all, sphingolipid and tyrosine metabolism increase additional risk factor in EAE severity with aging.

2873 The Mycotoxin Deoxynivalenol (DON) Facilitates Whey Sensitization in a Murine Food Allergy Model

M. Bol-Schoenmakers[3], S. Braber[3], P. Akbari[3], P. Jeurink[1], P. de Graaff[3], J. J. Smit[3], B. van Esch[3], J. Garssen[1,3], J. Fink-Gremmels[3] and R. H. Pieters[3]. *[1]Nutricia Research, Utrecht, Netherlands; [2]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands and [3]Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands.*

The mycotoxin deoxynyvalenol (DON) is among the most frequently detected contaminants of cereal-based foods, and mainly investigated for its cytotoxic and genotoxic effect. However, DON might also contribute to allergic sensitization to food proteins due to the ability to impair intestinal barrier function. Increasing evidence suggests an important role for epithelial cell stress or damage in initiating allergic sensitization at mucosal surfaces. The present study therefore investigated whether oral exposure of mice to DON together with whey resulted in allergic responses to this food protein. Female C3H/HeOuJ mice (n=8 per group) that were orally exposed to DON plus whey once a week for 5 weeks had increased levels of whey-specific IgG1, IgG2a and IgE in serum compared to control mice, treated with DON or whey alone. Furthermore, DON plus whey exposed mice showed an acute allergic skin response upon intradermal whey challenge. Analysis of intestinal tissues, isolated 6h after a single oral exposure to DON, revealed increased mRNA expression of the tight junction molecules claudin-2 and -3 and the adherens molecule E-cadherin. An observed increase in intestinal IL-33 mRNA was accompanied by an enrichment in innate lymphoid cells type 2 (ILC2) in the lamina propria after 24h in DON exposed mice. Moreover, whey-sensitized mice showed enhanced levels of soluble IL-33 receptor ST2 in serum from two weeks after initial exposure. Together, these results demonstrate that DON facilitates allergic sensitization, possibly via the local activation of IL-33 after epithelial cell stress. Our data therefore illustrate the possible contribution of food contaminants, like DON, in allergic sensitization in humans.

2874 Silica-Induced Autoimmunity Is Independent of Caspase-1

R. D. Pawar[4], J. Mayeux[4], A. S. Awad[3], T. K. Cooper[3], D. H. Kono[4] and K. M. Pollard[4]. *[1]Dept. of Comparative Medicine, Penn State University, College of Medicine, Hershey, PA; [2]Dept. of Immunology, The Scripps Research Institute, La Jolla, CA; [3]Dept. of Medicine, Penn State University, College of Medicine, Hershey, PA and [4]Dept. of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA.* Sponsor: K. M. Pollard.

Numerous environmental exposures have been associated with autoimmune disease in humans, of which one of the strongest is the association of occupational exposure to silica dust with systemic autoimmune diseases such as systemic lupus erythematosus. While the mechanisms responsible remain to be elucidated, inflammatory responses in the lung are likely a critical factor. Interleukin-1β (IL-1β), a pro-inflammatory cytokine and key mediator of inflammatory processes, plays a major role in silica-induced lung inflammation and pathology. IL-1β is initially synthesized as a pro-IL-1β form that is activated following caspase-1-mediated cleavage. To analyze the role of IL-1β in silica-induced autoimmunity, we exposed B10.S and Caspase-1 deficient B10.S (B10.S-Casp1-/-) mice to crystalline silica (1mg) in PBS or PBS only, by intranasal instillation twice a week for 4 weeks. Serum autoantibodies, analyzed at 28 weeks post-exposure, revealed that both B10.S and B10.S-Casp1-/- mice developed elevated levels of anti-chromatin and anti-nuclear antibodies compared to PBS controls. Histopathology analysis of the lungs revealed that silica treated B10.S and B10.S-Casp1-/- mice had a significant peribronchial and perivascular lymphocytic infiltrates, alveolar proteinosis, and alveolitis, compared to PBS controls. Total protein and cell numbers in broncho-alveolar lavage (BAL) fluid were also significantly greater in B10.S and B10.S-Casp1-/- mice. Infiltrating inflammatory M1 macrophages (CD11b+Ly6chi) and neutrophils (Ly6Ghi) were significantly increased in BAL fluid of B10.S and B10.S-Casp1-/- mice. These findings argue that caspase-1 is not solely required for for silica-induced autoimmunity in mice, but that other mechanisms possibly mediated through caspase-8, NLRP3 inflammasome, cathepsin C, and other IL-1 cleaving proteases (elastase, calpain, granzyme B, mast cell chymase) contribute.

2875 Broad Spectrum Antibiotic Treatment Enhanced T Cell Mediated Inflammation and Guillain Barré-Associated Antibody Responses to *Campylobacter jejuni* in a Mouse Model

P. T. Brooks[2], J. A. Bell[2], A. Malik[2] and L. S. Mansfield[2]. *[1]Comparative Enteric Diseases Laboratory, Michigan State University, East Lansing, MI and [2]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI.* Sponsor: A. Colón-Rodriguez.

Campylobacter jejuni is a leading cause of bacterial diarrheal illness worldwide. Disease is most often self-limiting; however, in approximately 7 in 10,000 infected individuals the autoimmune neurological diseases Guillain-Barré Syndrome (GBS) or its variant Miller Fisher Syndrome arise. When GBS is preceded by *C. jejuni* infection, lipooligosaccharides resembling host gangliosides activate the immune system to produce neurotoxic anti-ganglioside antibodies. Our aim was to determine the role of the gut microbiota in disease following infection with two *C. jejuni* GBS associated strains in a murine model. We hypothesized that alteration of the microbiota with a broad spectrum antibiotic, Cefoperazone (CPZ), would increase *C. jejuni* colonization, increase plasma anti-ganglioside antibodies, and increase disease severity compared to untreated controls. C57BL/6 IL-10-/- mice were administered antibiotic-free water or 0.5 mg/mL CPZ in their drinking water for 7 days prior to inoculation with the vehicle, *C. jejuni* 260.94 or *C. jejuni* D8942. Both bacterial strains are resistant to CPZ, allowing continued CPZ administration for 35 days post-infection when the mice were sacrificed. CPZ treatment enhanced T cell (CD19- CD3+) and T helper (TH) cell (CD19- CD3+ CD4+) responses in the colon of *C. jejuni* 260.94 and D8942 infected mice. Anti-ganglioside (anti-GM1 and -GD1a) and -*C. jejuni* antibodies were elevated in infected mice and these responses were exacerbated by CPZ treatment. GBS is the world's leading cause of acute neuropathy, and *C. jejuni* is the leading antecedent infection. However, not all enteritis cases due to *C. jejuni* strains bearing LOS that mimics nerve gangliosides result in GBS. Our results indicate that antibiotic treatment alters the immune response to *C. jejuni* in a way that may promote GBS. Projects were funded in whole with federal funds from NIAID, NIH, Department of Health and Human Services under ERIN CRC grant U19AI090872.

2877 Transmembrane, But Not Soluble, TNF Impairs CD4 TH17 T Cell Differentiation by Promoting IL-2 Production

P. Miller, M. Bonn and S. McKarns. *University of Missouri School of Medicine, Columbia, MO.*

Cytokines are key regulators of the differentiation of CD4 T helper cells into distinct subsets, including IL-17-producing TH17 cells. While IL-17 is critical for host defense, its overabundance promotes autoimmunity. Environmental factors have been implicated in the increased incidence of autoimmune diseases, including those involving the IL-23/IL-17 axis, over the past three decades. Here, we report elevated TNF receptor type 2 (TNFR2) expression in TH17 T cells. We further utilize genetic loss-of-function models as well as selective TNF blockade in wild-type to mice demonstrate that impaired transmembrane TNF/TNFR2 signaling, but not soluble TNF/TNFR1 signaling, promotes TH17 differentiation *in vivo* and *in vitro*. Under TH17 polarizing conditions, elevated IL-17 production by TNFR2 KO CD4+ T cells associated with upregulated STAT3 activity and downregulated STAT5 activity. Moreover, increased IL-17 production in TNFR2 KO mice was abrogated by addition of exogenous IL-2 to implicate an IL-2-dependent mechanism of action. We conclude that CD4+ T cell-intrinsic transmembrane TNF/TNFR2 promotes IL-2 production that inhibits the generation of TH17 cells in a FoxP3-independent manner. Moreover, under TH17 polarizing conditions, selective blockade of CD4+ T cell-intrinsic TNFR2 appears to be sufficient to promote TH17 differentiation. (This work is supported by NIH R01 ES022966).

2878 TNFR2 Deficiency Modulates Gut Microbiota to Promote CNS Autoimmune Disease

P. Miller, M. Bonn and S. McKarns. *University of Missouri School of Medicine, Columbia, MO.*

The mechanisms by which the environment influence human autoimmunity remain largely unknown. It is well established that tumor necrosis factor alpha (TNF) antagonism benefits patients with inflammatory autoimmune disorders such as Crohn's disease and rheumatoid arthritis, but unexplainably exacerbates CNS autoimmunity, including multiple sclerosis (MS). We now report that TNF receptor type 2 (TNFR2) deficiency promotes female-biased spontaneous autoimmune CNS demyelination in myelin oligodendrocyte glycoprotein-specific 2D2 T cell receptor transgenic mice. Oral antibiotic treatment prevents spontaneous autoimmunity in C57BL/6 TNFR2-/- 2D2 mice to suggest a role for gut microbiota. Illumina sequencing of fecal 16S rRNA identified a distinct microbiota profile in male TNFR2-/- 2D2 that associated with disease protection. *Akkermansia muciniphila, Sutterella sp., Oscillospira sp., Bacteroides acidifaciens,* and *Anaeroplasma sp.* were selectively more abundant in male TNFR2-/- 2D2 mice. In contrast, *Bacteroides sp., Bacteroides uniformis,* and *Parabacteroides sp.* were more abundant in affected female TNFR2-/- 2D2 mice suggesting a role in disease causation. Overall, TNFR2 blockade appears to disrupt commensal bacteria-host immune symbiosis to reveal autoimmune demyelination in genetically susceptible mice. Under this paradigm, microbes likely contribute to an individual's response to anti-TNF therapy. This model provides a foundation for host immune-microbiota directed measures for the prevention and treatment of CNS-demyelinating autoimmune disorders. (This work is supported by NIH R01 ES022966).

2879 Inhibition of Connective Tissue Growth Factor Attenuates Paraquat-Induced Lung Fibrosis in a Human MRC-5 Cell Line

M. Huang. *Department of Occupational and Environmental Health Sciences, School of Public Health, Ningxia Medical University, Yinchuan, China.*

Chronic exposure to Paraquat (PQ) may result in progressive pulmonary fibrosis and subsequent chronic obstructive pulmonary malfunction. Connective tissue growth factor (CTGF) has been proposed as a key determinant in the development of lung fibrosis. We investigated thus whether knock down of CTGF can prevent human lung fibroblasts (MRC-5) activation and proliferation with the subsequent inhibition of PQ-induced fibrosis. MRC-5 was transfected with CTGF-siRNAs and exposed to different concentrations of PQ. The siRNA-silencing efficacy was evaluated using western blotting analyses, qRT-PCR and flow cytometry. Next, the viability and migration of MRC-5 was determined. MMP-2, MMP-9 and TIMP-1 accumulation were quantified to evaluate the lung fibrosis exposure to PQ. Over expression of CTGF mRNA was observed in human MRC-5 cell as early as 6 hours following PQ stimulation. CTGF gene expression in MRC-5 cells was substantially reduced by RNAi, which significantly suppressed the expression of the lung fibrosis markers such as tissue inhibitor of metalloproteinase-2 (TIMP-2), Matrix metalloproteinase-2 (MMP-2) and Matrix metalloproteinase-9 (MMP-9) that were stimulated by PQ. Inhibition of CTGF expression suppressed impeded the proliferation and migration ability of MRC-5 cells and resulted in cell-extracellular matrix (ECM) protein accumulation in cells. Our results suggest that CTGF promoted the development of PQ-induced lung fibrosis in collaboration with transforming growth factor β1 (TGFβ1). Furthermore, the observed arresting effects of CTGF knock down during this process suggested that CTGF is the potential target site for preventing PQ-induced pulmonary fibrosis (sponsored by National Science Foundation of China NO. 81360435).

2880 Chlorpyrifos Induces NLRP3 Inflammasome and Programmed Cell Death Through Mitochondrial Oxidative Stress in HaCaT, Human Keratinocyte

Y. Jang and M.-h. Cho. *Seoul national university, Seoul, Korea, Republic of.*

Chlorpyrifos (CPF) pesticide has been broadly applied around the world residentially as well as agriculturally. Although various researches have reported the toxicity and health effects derived by CPF exposure, the molecular mechanism of CPF toxicity to skin is not well-identified. The present study determined the potential mechanism involved in skin toxicity of CPF using human skin keratinocyte, HaCaT cell line. After treating to HaCaT cells, CPF triggered reactive oxygen species (ROS) and mitochondrial oxidative stress. We focused on NLRP3 inflammasome known to induce innate immune response. We used mitoTEMPO, a mitochondrial ROS (mROS) scavenger, to demonstrate the role of mROS in NLRP3 inflammasome and programmed cell death induced by CPF. Our results showed that CPF provoked NLRP3 inflammasome and pyroptosis/apoptosis through increase of mROS in HaCaT cells. This study clarified how CPF induced innate immune response and skin inflammation through activating NLRP3 inflammasome in skin keratinocytes. CPF may develop cutaneous disease and antioxidants could be proposed for a therapeutic agent to protect skin against exposure to CPF pesticide. Acknowledgements: Part of this work was supported by "Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of alternative test for systemic toxicity and network analysis of toxicity parameters, Project No. PJ00995002)" of Rural Development Administration, Republic of Korea.

2881 Physiological responses in *Caenorhabditis elegans* Exposed to Deltamethrin

P. Shen[3], Q. Sun[3], Y. Yue[3], Z. Gao[3], J. Yang[1], H. Xiao[3], J. M. Clark[3] and Y. Park[3]. [1]*Amherst Regional High School, Amherst, MA;* [2]*Department of Food Science, University of Massachusetts-Amherst, Amherst, MA and* [3]*Department of Veterinary and Animal Science, University of Massachusetts-Amherst, Amherst, MA.* Sponsor: Y. Park.

The objective of this study was to explore the long-term effects of deltamethrin on physiological functions (growth rate, pumping rate, reproduction, and locomotive activities), lifespan and fat accumulation of *C. elegans*. Deltamethrin (0.1, 1, 10 μM) did not show any effects on growth rate and pumping rate but significantly reduced the total progeny number (by 14%, 17% and 23%, respectively) and locomotive activities (by 35%, 44% and 48% on day-6, respectively). aak-2 (which encodes AMPKα subunit) was shown to be involved in this reduction of locomotive activities. Furthermore, 10 μM deltamethrin significantly decreased the median lifespan of *C. elegans* by 32% compared to the control group. This lifespan reduction was found to rely on age-1 and daf-16 via the insulin/IGF-1 signaling pathway. Deltamethrin at 10 μM also increased fat accumulation of *C. elegans* by 48% after a 7-day treatment. Our results demonstrate the long-term effects of deltamethrin on *C. elegans*, which may provide important information for obesity and locomotive studies in mammals.

2882 Safety Evaluation of Parastar® Plus in Dogs and Assessment of Transferable Residue of Fipronil and Cyphenothrin from Dogs to Humans. Is There a Health Concern?

K. Case, N. Vega, R. C. Gupta, M. Lasher and T. D. Canerdy. *Murray State University, Murray/Hopkinsville, KY.*

Currently, there are approximately 80 million dogs in the United States. The majority of these dogs are pets, while some are used in military and police K9 units. Recently, trained dogs have also been used for the early detection of cancer (breast, cervical, and prostate) and seizures in humans. Dogs are easily infested with fleas, ticks, and other ectoparasites, some of which may serve as vectors for transmitting bacterial, viral, and parasitic diseases, therefore, the use of ectoparasiticides is inevitable and important. The present investigation was undertaken with two specific objectives: one, to evaluate the safety of fipronil and cyphenothrin in dogs after topical application of Parastar® Plus; and two, to determine the transferable residue of these insecticides from dogs to humans. Six healthy adult dogs (medium length hair, and weighing between 45-60 pounds) received topical application of Parastar® Plus (2.68 mL; fipronil, 9.8% and cyphenothrin, 5.2%) on the back between the shoulder blades. At pre-determined intervals, dogs were given a full physical exam, and residues of fipronil and cyphenothrin were determined in dog blood and cotton gloves used to pet the dogs using GC/MS. Fipronil and cyphenothrin peaks eluted at 7.453 and 9.913 minutes, correspondingly, and the compounds were confirmed based on their characteristic ions. At no time was fipronil or cyphenothrin residue detected in blood samples. In glove extracts, residues of fipronil and cyphenothrin were maximally present at 24 hr post-treatment (43.84±5.69 and 59.26±2.45 μg/g, respectively). By 48 hr, the residue levels sharply declined (16.89±2.58 and 17.98±2.07 μg/g, respectively). The insecticides' residues were detected in insignificant amounts after one week (5.68±2.16 and 9.99±1.51 μg/g, respectively), and only in trace amounts after two weeks. At no time did any dog show any side effects, except itching at the site of Parastar® application. The findings suggest that Parastar® Plus is safe for dogs and transferable residues of fipronil and cyphenothrin are minimal, posing very little or no health concern to pet owners or veterinary personnel. Of course, veterinary personnel, who handle many dogs daily, may require proper protection to avoid cumulative exposure.

2883 Changes in Gut Microbiota May Be Early Signs of Liver Toxicity Induced by Epoxiconazole in Rats

Q. Liu, C. Xu and A. Gu. *Nanjing Medical University, Nanjing, China.* Sponsor: a. Gu.

Background: The gut microbiome is essential for human health due to its effects on disease development, drug metabolism and the immune system. It may also play a role in the interaction with environmental toxicants. However, the effect of epoxiconazole, a fungicide active ingredient from the class of azoles developed to protect crops, on the abundance and composition of the gut microbiome has never been studied. We put forward the hypothesis that changes in gut microbiota may be early signs of toxicity induced by epoxiconazole. Methods: In this study, female rats were fed with epoxiconazole-adulterated diets (0, 4 and 100 mg/kg/day) for 90 days. The gut microbiome was determined by 16S rRNA gene sequencing. Body and organ weight, and blood biochemistry were also measured after 90 days of oral epoxiconazole exposure. Results: Interestingly, the abundance of gut Firmicutes decreased, and Bacteroidetes and Proteobacteria increased. At family level, Lachnospiraceae and Enterobacteriaceae were selectively enriched following epoxiconazole exposure. Our results indicate that epoxiconazole exposure may induce changes in the gut microbiome and potential liver toxicity. Conclusion: Changes in the gut microbiome may be used as early indicators for monitoring the health risk of the host.

2884 Chlorpyrifos: Neurodevelopment, AChE Inhibition Weight of Evidence

M. Silva and S. Koshlukova. *Pesticide Regulation, California EPA, Sacramento, CA.*

The main mechanism of toxicity of the insecticide chlorpyrifos (CPF) is related to the ability of its oxon metabolite to bind and inhibit acetylcholinesterase (ChE) in the central and peripheral nervous systems. Concerns were raised about CPF regulatory standards based on its inhibitory effects on ChE activity that may overlook more sensitive non-cholinergic mechanisms. Increasing evidence points to CPF effects on the nervous system development in mammals and zebrafish (ZF) at exposures below those that inhibit ChE (ChEI). Noncholinergic mechanisms are suggested for CPF neurodevelopmental toxicity and this is important when deciding weight-of-evidence (WoE) in pesticide risk characterization. We reviewed *in vivo* data and the *in vitro* CPF Toxicity Forecaster (ToxCast) database to assess the WoE for noncholinergic effects at low CPF exposure. There were no true actives in ToxCast assay results (only cytotoxicity). *In vivo* studies with rat pups showed that CPF at 0.5 mg/kg/d disrupted CNS cannabinoid and dopamine metabolism, and induced behavioral effects in the absence of brain ChEI. Epidemiological data showed long-lasting deficits in working memory and effects on brain morphology in children aged 3-13 years after *in utero* CPF exposure. A PBPK-PD model is currently being used by regulatory agencies for estimating reference doses is based on 10% RBC AchEI from human studies. The estimated oral human Point of Departure (PoD) for steady state (21-day: most relevant scenario for *in utero* human) exposure was 780 ng/kg. Others used this PBPK-PD model with the same human data to estimate the CPF maternal exposure that would by necessary to produce decrements in their child's working memory at 7 yr. From these estimates the oral *in utero* PoD for working memory decrements was 3.4-fold lower than the 780 ng/kg predicted by the PBPK-PD model for 10% RBC ChEI. Supporting mammalian/human data, many ZF studies showed irreversible neurobehavioral deficits at 10-fold lower exposures (0.01 uM) than those inhibiting ChE (0.10 uM). These results provide WoE that the pathways impacted by CPF in the developing brain may be independent of ChEI.

2885 Changes Induced by Fipronil Sulfone Metabolite in Oxidative Stress Biomarkers in SH-SY5Y Cells and Protective Effect of Tempol

A. Anadón, M. A. Martínez, I. Ares, E. Ramos, M. Martínez, V. Castellano, M. R. Martínez-Larrañaga and A. Romero. *Department of Toxicology and Pharmacology, Complutense University, Madrid, Spain.*

There is substantial evidence indicating that oxidative stress increased during pesticide poisoning. Oxidative stress generated by pesticides could be one of the factors underlying the pathogenesis of neurodegenerative disorders. Fipronil, an N-phenylpyrazole with a trifluoromethylsulfinyl substituent, is widely used in agriculture, termite/fire ant control, and in pet ectoparasite treatment. Fipronil acts on GABAA receptors as a noncompetitive blocker of the GABA-gated chloride channel. Exists evidence that fipronil may also cause neuronal cell toxicity through a different pathways than the primary target GABAA receptors. Studies showed that fipronil caused neuronal toxicity in SH-SY5Y cells via an apoptotic pathway initiated by reactive oxygen species. *In vivo* mammalian studies indicate that primary metabolic pathway for fipronil involves oxidative formation of the sulfone metabolite. Although some aspects about of fipronil to be toxic in human cells have been reported, results about the sulfone metabolite neurotoxicity are limited. Herein, we report that fipronil sulfone metabolite induces changes in oxidative stress biomarkers following exposure in a dopaminergic cell model, SH-SY5Y cells. Lipid peroxidation was estimated by the thiobarbiturate test for malonaldehyde (MDA) and nitrite production (NO). Our results demonstrated that fipronil sulfone (3 μM) induced a significant increase in MDA levels (5.0-fold, $p<0.001$) compared to control group as well as induced a significant increase in NO levels (2.0-fold, $p<0.001$) compared to control group. Tempol, a superoxide dismutase mimic with additional reactive oxygen species-scavenging activities, completely reversed the decrease in SH-SY5Y cell viability caused by fipronil sulfone metabolite. This work was supported by Project Ref. UCM-BSGH/ GR3/14 from Universidad Complutense de Madrid, and Project (ALIBIRD-CM Program) Ref. S2013/ ABI-2728 from Comunidad de Madrid.

2886 Esterase Detoxification of Acetylcholinesterase Inhibitors by Human Liver Samples *In Vitro*

V. Moser[2] and S. Padilla[2]. [1]*ISTD, US EPA, Research Triangle Park, NC and* [2]*TAD, US EPA, Research Triangle Park, NC.*

Organophosphate (OP) and N-methylcarbamate pesticides inhibit acetylcholinesterase (AChE), but differences in metabolism and detoxification can influence potency of these pesticides across and within species. Carboxylesterase (CaE) and A-esterase (paraoxonase, PON) are considered factors underlying age-related sensitivity differences. We used an *in vitro* system to measure detoxification of AChE-inhibiting pesticides mediated via these esterases. Recombinant human AChE was used as a bioassay of inhibitor concentration following incubation with detoxifying tissue: liver plus Ca+2 (to stimulate PONs, measuring activity of both esterases) or EGTA (to inhibit PONs, thereby measuring CaE activity). Inhibitory concentrations of aldicarb, chlorpyrifos oxon, malaoxon, methamidophos, oxamyl, paraoxon, and methyl paraoxon were incubated with liver from adult male rat or one of 20 commercially provided human (11-83 years of age) liver samples. Detoxification was the difference in inhibition produced by the pesticide alone or in combination with liver plus Ca+2 or EGTA. Generally, rat liver produced more detoxification than did the human samples. There were large detoxification differences, which were mostly not correlated with age or sex, across human samples for some pesticides (especially malaoxon, chlorpyrifos oxon) but not for others (e.g., aldicarb, methamidophos). Chlorpyrifos oxon was fully detoxified only in the presence of Ca+2 in both rat and human livers. Detoxification of paraoxon and methyl paraoxon in rat liver was greater with Ca+2, but humans showed less differentiation between Ca+2 and EGTA conditions. This suggests the importance of PON detoxification for these three OPs in the rat, but mostly only for chlorpyrifos oxon in human samples. Malaoxon was detoxified similarly with Ca+2 or EGTA, and the differences across humans correlated with metabolism of p-nitrophenyl acetate, a substrate for CaEs. This suggests the importance of CaEs in malaoxon detoxification. Understanding these individual differences in detoxification can inform human variability in pesticide sensitivity. This abstract may not necessarily reflect official Agency policy

2887 Proteomic Evaluation of Diisopropylfluorophosphate (DFP) Sub-Acute Non-Cholinergic Effects in Rat Hippocampus

A. Hoffmann[1,2], D. A. Mahle[1], M. L. Meade[1], M. K. Makley[1,2] and J. M. Gearhart[1,2]. [1]*Molecular Bioeffects Branch, Bioeffects Division, Human Effectiveness Directorate, 711th Human Performance Wing, Air Force Research Laboratory, Wright Patterson AFB, Dayton, OH and* [2]*The Henry M Jackson Foundation for the Advancement of Military Medicine, Dayton, OH.*

Organophosphate (OP) pesticide exposure represents a concern for the human population. While the acute effects of OPs are based on acetylcholinesterase inhibition, the non-cholinergic sub-acute effects are relatively unknown. This study uses broad-spectrum proteomic analysis to identify hippocampal non-cholinergic OP effects following rat diisopropylfluorophosphate (DFP) exposure. Adult male Fischer 344 rats (n=29 total) were subcutaneously administered a one-time subacute dose of 1mg/kg DFP (n=14) or saline (n=15), and hippocampal tissue effects were analyzed 2, 12 or 24h after exposure. Hippocampal tissue was lysed and processed by tryptic digest and tandem mass tags (TMT) labeling. Samples were separated on a Waters NanoUPLC prior to Thermo LTQ Orbitrap Velos analysis (LC-MS/MS). TMT LC-MS/MS SEQUEST relative peptide quantitation of different exposure time points

detected between 1448 and 2123 EnsemblProt identifiers that matched between 581 to 962 WikiGene identifiers. Cytoscape3.1.1 pathway analysis using built-in applications Reactome FI 4.1.1beta, ClueGo2.1.5 and Metscape3.1.1 demonstrated only marginal changes in protein expression at the 2h time point related to cell adhesion, targeted protein degradation, ATP-dependent calcium ion transport, metabotropic glutamate receptor signaling, innate immune response, and neuronal differentiation. Twelve-hour DFP exposure resulted in limited changes with regards to calcium binding/signaling, intracellular transport, cellular structural proteins, cell division and epithelial-to-mesenchymal transition with an upregulation of glutathione S-transferase. Twenty-four hour DFP exposure demonstrated more prominent changes with regards to calcium overload compensation by modulation of calcium binding/signaling, synaptic transmission including a downregulation of the ITPR1 receptor. Further protein modulations were related to vesicle transport, cytochrome C energy metabolism, cytoskeletal and extracellular organization, immune response, targeted protein degradation by ubiquitin, DNA repair/chromatin remodeling and cell cycle.

PS 2888 Thiuram Early Life Stage Toxicity Extends into More Detailed Alterations in Older Individuals: A Case for Thyroid Involvement?

X. Chen[1], M. Fang[3], M. Chernick[3], F. Wang[1], J. Yang[1], Y. Yu[1], N. Zheng[2], H. Teraoka[4], S. Nanba[4], T. Hiraga[4], H. M. Stapleton[3], D. E. Hinton[3] and W. Dong[1,3]. *[1]Inner Mongolia Provincial Key Laboratory for Toxicants and Animal Disease/Inner Mongolia University for the Nationalities, Tongliao, China; [2]Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agricultural Ecology, Chinese Academy of Sciences, Changchun, China; [3]Nicholas School of the Environment, Duke University, Durham, NC and [4]School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan*. Sponsor: W. Dong.

Thiuram, a pesticide of the dithiocarbamate chemical family, is widely used to prevent fungal disease in seeds and crops. The off-site movement of this pesticide to surface waters occurs; however, to date, little toxicological information is available, and residual toxicity as well as aquatic toxicity to early life stage fish is of environmental concern. Previously, we found exposure to thiuram caused developmental toxicity possibly attributable to thyroid hormone disruption. In the present study, zebrafish embryos were used to investigate thiuram developmental toxicity after acute (1 hour) exposure (0.001 μM to Y 10 μM) at different development stages. Next, organisms were observed from 24 to 120 hours-post-fertilization (hpf). Embryo survival was decreased at 1 μM and 10 μM and hatching rate was delayed at 0.1 μM and 1 μM. The presence of notochord curvatures, a common biomarker in previous studies, was significantly increased at 0.1 and 1 μM thiuram exposure of 1 hour duration to embryos at stage 2 and 10 hpf. In contrast, no obvious changes were observed for those embryos dosed at later stages (i.e., > 24 hpf). Expression of Deiodinase 3, an important thyroid enzyme in embryonic development and metamorphosis, was significantly increased at 24 hpf in individuals exposed to 0.1 or 1 μM thiuram at 10 hpf. Thyroid peroxide (TPO), an important enzyme in T4 synthesis, was significantly decreased at 96 hpf by 1 μM thiuram exposure of embryos at 10 hpf. A similar notochord curvature was observed using TPO inhibitor methimazole (MMI). Toxicity of thiuram is stage-dependent and early life stage exposure could be responsible for adverse effects later in life. Adverse effects may be due to impacts on the thyroid system through the regulation of specific thyroid genes including TPO and Deiodinase 3.

PS 2889 Exposure to Permethrin Increases Body Fat Mass and Alters Glucose Metabolism in Response to High Fat Diet in Male C57BL/6J Mice

X. Xiao[4], Q. Sun[4], Y. Kim[4], D. Kim[4], K. S. Yoon[1], J. M. Clark[4] and Y. Park[4]. *[1]Department of Biological Sciences and Environmental Sciences Program, Southern Illinois University, Edwardsville, IL; [2]Department of Food Science, University of Massachusetts, Amherst, MA; [3]Department of Mathematics and Statistics, University of Massachusetts, Amherst, MA and [4]Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA*.

Permethrin, a pyrethroid pesticide, was previously reported to promote adipogenesis and worsen insulin resistance *in vitro*. Earlier *in vivo* studies on female mice also indicated that permethrin, interacting with dietary fat, could induce insulin resistance. This study investigated the interaction between permethrin exposure and dietary fat on development of obesity and diabetes in male mice. We tested three different doses of permethin (0.05, 0.5, & 5 mg/kg body weight/day) in low-fat-fed (4% w/w of diet) or high-fat-fed (20% w/w of diet) male C57BL/6J mice for 12 weeks. Body weight and food intake were measured weekly. Insulin resistance was measured by insulin tolerance test (ITT), glucose tolerance test (GTT), serum insulin concentrations and Homeostatic model assessment (HOMA). Our results showed that permethrin treatment significantly increased body weight and fat mass in high-fat fed groups without significantly influencing food intake, but no significant effect were observed in low-fat fed animals. Animals fed permethrin in high-fat diet significantly increased insulin resistance, as indicated by ITT (0.5 & 5 mg/kg/d), GTT (5 mg/kg/d), and HOMA (5 mg/kg/d). Serum analysis further showed that permethrin treatment (5 mg/kg) significantly elevated blood level of glucose, cholesterol and leptin in high-fat groups, but not in low-fat fed groups. These results suggest that there are significant interactions between dietary fat content and permethrin in altered lipid and glucose metabolism. Exposure to permethrin in combination of high fat diet may contribute to develop obesity and type 2 diabetes *in vivo*. This project is supported by NIH R21ES023676

PS 2890 Maneb and Mancozeb Induce Senescence via p53, p27 and p15/16 Pathways

M. Metodieva[2], N. Simone[1], J. Seo[2], C. Ta[2] and S.-Y. Cheng[2]. *[1]Holy Cross High School, Queens, NY and [2]Sciences, John Jay College of Criminal Justice, New YORK, NY*.

Maneb (MB) and mancozeb (MZ), which belong to the ethylene-bis-dithio-carbamates (EBDCs) family, are widely used as fungicide. It is a polymer chemical complex containing manganese and/or zinc ions. Studies showed that MB and MZ induce DNA damage and activate NF-kappa B signaling pathway. Cellular senescence is an irreversible cell cycle arrest in response to DNA damage and oxidative stress. Activation of NF-kappa B signaling pathway has a causal role in promoting senescence. The data obtained from Cheng's lab showed MB and MZ induced senescence in PC12 cells. An increase in senescence biomarker SA-β-Gal staining was observed from PC12 cells that were given 4 hours of MB and MZ treatments (10-50 μM) and 24 hours of recovery. The increase in SA-β-Gal staining was more perceptible in cells treated with low dose (10 μM) as compared to the cells treated with higher doses (20 and 50 μM). The quantitative data of SA-β-Gal were coherent with SA-β-Gal staining results. Moreover, our study showed that PC12 cell proliferation was halted at G1/G0 phase after 24 hours of 50 μM MB and 50 μM MZ exposures. This study was also conducted to reveal the signaling pathway which may be involved in these toxic responses of MB and MZ. PC12 cells were treated with various concentrations of MB and MZ (5-50 μM) for 24 hours. After chemical treatments, cells were either subjected to Western blot analysis, to semi-quantify the expression of p53, p27, and p15/16, or immunocytochemistry. The results showed that the expressions of p53, p27 and p15/16 were increased in PC12 cells treated with MZ and MB in a dose-dependent manner. This study demonstrates that MB and MZ can trigger cell cycle arrest and cellular senescence and increase the expression of p53, p27, and p15/16.

PS 2891 Evaluation of Growth and Plasma Membrane H+-ATPase Inhibition in *Candida albicans* by Selected Sulfur Analogs of Ebselen

N. N. Orie[2], M. Pietka-Ottlik[1] and B. Billack[2]. *[1]Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland and [2]Pharmaceutical Health Sciences, St. John's University, Jamaica, NY*.

Our laboratory has previously shown that the yeast plasma membrane H+-ATPase (Pma1p) is at least one of the targets responsible for the antimycotic activity of ebselen (EB), an organoselenium compound with noted antiinflammatory and antioxidant properties. In the present study, EB-1S and EB-2S, two sulfur analogs of EB, were investigated for antifungal activity. To this end, liquid suspensions of the FLU-resistant strain S2 were prepared and the effect of the test compounds upon growth was individually assessed using a microbroth dilution method in RPMI 1640 medium. After a 48 h incubation of yeast cells with each respective test compound, EB-1S was observed to be less active than EB, while EB-2S exhibited an antifungal activity similar to that of EB. In additional studies with a second FLU-resistant strain, ATCC 96901, EB-2S was found to be more potent at inhibiting the growth of yeast cells than either EB or EB-1S. To determine whether Pma1p plays a role in the antimycotic activity of the organosulfur test compounds, the effect of said compounds upon medium acidification was evaluated using intact S2 cells and isolated Pma1p enzyme. Compared to EB, the sulfur analogs exhibited only slight inhibition upon medium acidification by intact cells, but a similar and concentration-dependent inhibition on ATPase activity measured from a partially purified membrane preparation of S2

cells. Taken together, these data indicate that EB-1S and EB-2S inhibit the growth of FLU-resistant strains of *C. albicans*, in a manner consistent with blocking Pma1p. Moreover, EB-1S and EB-2S may be worthy of future study in the search for novel antifungal agents which are needed to combat fungal infections exhibiting resistance to azoles.

2892 Embryonic Exposure to Zinc dimethyldithiocarbamate Alters Notochord Development in Japanese Medaka

A. T. Watson, I. T. Stancil and S. W. Kullman. *Biological Sciences, North Carolina State University, Raleigh, NC.*

Zinc dimethyldithiocarbamate (Ziram) is a current use pesticide with known teratogenic and skeletal effects on multiple model organisms *in vivo*. In this study, Ziram was assessed for its potential to cause skeletal deficits following embryonic exposure in Japanese medaka (Oryzias latipes), a small teleost fish model of human skeletal development. Orange-red hd-dR (wildtype) embryos were exposed to Ziram (0.01-10 µM) and individuals were assessed at 10 dpf for skeletal deficits, histological staining of bone and cartilage, and expression of key osteochondral genes. Ziram-exposed fish demonstrate severe lordosis, kinking of the notochord, and a reduction in total body length. To explain these phenotypes, it was hypothesized that Ziram interferes with early vacuolization of the notochordal sheath cells. Preliminary results indicate that exposure to Ziram causes a dose-dependent reduction in rab32a expression, a key regulator involved in late endosomal trafficking necessary in formation of notochord vacuoles. This finding corroborates other studies that suggest a loss of rab32a prevents vacuolization of notochord cells and subsequent elongation of the body axis and spine formation. Current work is focused on investigating other components of the endosomal trafficking pathway, and will dissect the molecular initiating events that modulate Ziram-mediated reduction of rab32a.

2893 Applying the 3R's in Regulatory Testing: Use of the Developmental Toxicity Limit Test to Reduce Animal Use in Development of a Novel Agrochemical, Rinskor™

S. Papineni[1], B. R. Hannas[2], L. A. Murphy[2], M. P. Bell[2], V. A. Marshall[2] and R. J. Rasoulpour[1]. [1]*Dow AgroSciences, LLC, Indianapolis, IN and* [2]*The Dow Chemical Company, Midland, MI.*

Development and registration of novel plant protection products requires a multi-year toxicity testing program for human health risk assessments. Global regulatory requirements stipulate a wide array of studies to evaluate end points such as developmental and reproductive toxicity which place a large demand on animal usage. In an effort to provide a significant reduction in animal use as promoted under the principles of the 3R's, we implemented the 'limit test' as defined by the OECD 414 and US EPA OPPTS 870.3700/Prenatal Developmental Toxicity Study guidelines for a novel herbicide, Rinskor™ active (Rinskor). Use of the limit test was based on the absence of toxicity at the limit dose (1000 mg/kg/day) in preceding at and rabbit reproductive and developmental probe studies for this ompound. This approach eliminated the use of low and mid-dose groups, thereby reducing animal numbers by 48 dams (rat study) and 48 does (rabbit study) plus their respective litters for a total saving of approximately 1,000 animals. Based on this methodology, two dose groups of 0 or 14,000 ppm (time weighted average of 0 or 975 mg/kg/d) in the rat and 0 or 27,000 ppm (time weighted average of 0 or 1042 mg/kg/d) in the rabbit epresenting the 'limit tests' were conducted. This approach was further supported by previous toxicokinetics data generated in preceding rat and rabbit reproductive and developmental probe studies indicating blood levels of its major acid metabolite to be nearly equivalent between the low- and high-dose groups indicating sublinear kinetics with administration of Rinskor. In both studies, administration of the active ingredient in the diet at the limit dose produced no treatment-related maternal toxicity and no indication of embryo/fetal toxicity or teratogenicity. These results and consideration of the number of animals saved emphasizes the utility of limiting the number of dose groups by implementing the limit test approach in this case study.

2894 Atrazine's Cytotoxicity in Immortalized Cell Lines

T. S. Blau, B. Narayanan and M. Costa. *Department of Environmental Medicine, NYU Langone Medical Center, Tuxedo Park, NY.*

Introduction: Atrazine (ATZ) is one of the most widely used herbicides in the U.S. with approximately 75-82 million lbs. applied per-year. The herbicide inhibits electron transport during photosynthesis in broadleaf weeds and induces oxidative stress. Due to its pervasiveness, the E.U. banned its use in 2004. In the U.S., ATZ remains one the most ubiquitous groundwater contaminants. Hypothesis: While ATZ is known to stifle weed growth by killing plant cells, little is known about the effect of the herbicide on human cells. Although plants and human cells differ, both possess similar electron transport systems. Based on this fact and literature that ATZ may have cytotoxic effects on human lymphocytes, we investigated whether treating immortalized cell lines might conclude similar results, and what the cytotoxic mechanism might be. We surmised that if our hypothesis proved correct, the resultant cell death might be facilitated by oxidative stress and downstream production of inflammatory cytokines and transcription factors. Results: HEK-293, BEAS-2B, A549, and PTEN-CaP 8 cells were treated, at varying doses (5-100 ug) and timelines (24-72 hrs) with the herbicide and assayed for cell viability using an MTT. Low doses of ATZ induced moderate increased cell viability (> 2 fold), whereas higher doses, (40-50 ug), yielded substantially decreased viability (> 4 fold). We then treated HEK-293 cells with the threshold dose (40 ug) and used flow cytometry for analysis of cellular DNA content. Triplicates of this assay showed a significant decline in DNA synthesis and cell proliferation. Production of reactive oxygen species (ROS) was then measured in all cell lines using DCFDA. All treated cell lines exhibited ROS production, but A549 cells produced the most significant increase. Conclusion: Due to ATZ's prevalent and growing use in the U.S. and banning in the E.U., we rationalize that this investigation is valuable and may be telling of possible public health outcomes at both the general population level (low dose) and at the occupational health level (high dose).

2895 Investigation of Essential Metal Accumulation in Rat after Oral Mancozeb Exposure

B. Kistinger and D. Hardej. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Mancozeb belongs to the ethylene bisdithiocarbamate class of fungicides which also include Maneb and Zineb. Mancozeb is used in the United States for its broad range efficacy against fungus on fruit, nut, and vegetable crops. The organic backbone of Mancozeb is complexed with both manganese and zinc metal ions. Recently our lab has shown Mancozeb to be toxic to gastrointestinal cells and this toxicity was coupled with the accumulation of the metal moieties within the cell. The focus of this study was to determine if Mancozeb can influence metal homeostasis *in vivo*. Male Sprague-Dawley rats (8 weeks old) were dosed by oral gavage for 28 consecutive days with either 100 mg/kg Mancozeb or vehicle control (50% v/v PEG 400). Animals were sacrificed on Day 29 and serum chemistry and tissue metal accumulation were analyzed. Serum chemistry was assayed immediately after animals were sacrificed using Abaxis VS2 Comprehensive Diagnostic Profile. A significant decrease in serum alkaline phosphatase levels was observed [122.0 ± 12.57 Units/L] compared to control [184.0 ± 21.22 Units/L]. Tissue accumulation of metals was measured using Inductively-Coupled Plasma Optical Emission Spectroscopy (ICP-OES). In the liver, no significant changes were observed in manganese levels between control [1.382 ± 0.01608 ppm/Dry Weight (g)] and treated [1.382 ± 0.04882 ppm/Dry Weight (g)] groups. There was also no significant difference in liver zinc metal levels between control [18.07 ± 0.3694 ppm/Dry Weight (g)] and treated [17.38 ± 0.2832 ppm/Dry Weight (g)] groups. In the kidney, manganese levels were significantly increased after Mancozeb treatment [0.7279 ± 0.01404 ppm/Dry Weight (g)] compared to control [0.6642 ± 0.01265 ppm/Dry Weight (g)]. In contrast, the level of zinc in the kidney was statistically lower in the treated group [14.67 ± 0.2238 ppm/Dry Weight (g)] compared to controls [15.82 ± 0.1447 ppm/Dry Weight (g)] . Further research is being done on the metal accumulation profiles in other organs and possible connection to oxidative stress.

2896 Epigenetic Regulation of Chlorpyrifos Induced Adipogenesis Using 3T3-L1 *In Vitro* Model

L. Yin, T. Mowla and X. Yu. *Environment Health Science, University of Georgia, Athens, GA.*

The accumulated evidences have suggested that environmental exposures contribute an additional risk factor for the development of obesity. Chlorpyrifos (CPF), one of the most widely used organophosphate (OP) insecticides, has been shown the potential developmental neurotoxicity. Our previous studies showed that CPF induced a significant disturbance in lipid metabolism and insulin pathways in the offspring when CPF exposed to the maternal gestational rats. However, the underline molecular mechanism of CPF involved in adipogenesis remains unclear. The goal of this study aims to determine the effect of CPF exposure on adipogenic signaling pathways and epigenetic regulation at the molecular level and decipher CPF's role in the development of obesity using 3T3-L1 *in vitro* model. We utilized high content and multiplexed cellomics assays (ArrayScan VTII, Thermo Scientific), and quantitatively analyze cell-based imaging (Bio Activation Studio) with neutral lipid droplets stained with LipdTOX Green (Life Technology). The results showed that CPF induced significant increases in number, the size and the intensity of lipid droplets with CPF at 10 μM and above. Using quantitative real-time RT-PCR, it was determined that CPF treatment significantly affected adipogenic transcriptional factors PPARγ and C/EBPα and adipocyte maturation marker LPL and FASN in a dose-dependent manner. We further examined the role of the epigenetic regulation in CPF-induced gene alterations in the adipogenic process. Cells pre-treated with the histone deacetylases (HDAC) inhibitor trichostatin A (TSA), or DNA methyltransferases (DNMTs) inhibitor 5-azacytidine (AZA) significantly altered the CPF-induced adipogenic gene expression, showing time-specific patterns. The data suggest that epigenetic modification may be involved in CPF-induced adipogenesis. In conclusion, our data strongly supports that CPF at doses without overt toxicity may potentially affect obesity development and that epigenetic regulation may be involved in this process (supported by R21 OH 010473, ARDF and the UGA Startup Research funding).

2897 Quantitation of cis- and Trans-Permethrin in Rat Plasma Using Gas Chromatography Negative Chemical Ionization Mass Spectrometry (GC-NCI-MS)

D. Gullick[3], J. V. Bruckner[3], M. D. Krolski[1], D. Gammon[2], C. A. White[3], B. S. Cummings[3] and M. G. Bartlett[3]. [1]*Bayer Crop Science, Research Triangle Park, NC;* [2]*FMC CORP, Ewing, NJ and* [3]*University of Georgia, Athens, GA.*

Trace analysis of pyrethroids in biological samples is often necessary for environmental risk assessments. To date, highly sensitive methods to measure pyrethroids have relied on availability of large samples related to environmental exposure (water, soil, food) or urine. There are no validated methods in the literature for determining this class of compounds in small (100 uL) plasma samples. In order to obtain data for construction and validation of physiologically-based toxicokinetic models, highly reproducible and sensitive bioanalytical methods are needed. Cis- and trans-permethrin were extracted from homogenated plasma samples by protein precipitation using acetonitrile and phosphoric acid. Deltamethrin was used as the internal standard. The samples were vortexed and centrifuged before evaporation to dryness. Extracted pyrethroids were reconstituted and extracted from the residue using toluene, prior to injection into an Agilent Model 6890N gas chromatograph equipped with a Model 5973 quadrupole mass analyzer. Samples were ionized via electron capture in the negative ion mode using methane as a chemical ionization gas. Fragment ions were monitored using selected-ion monitoring for quantitation and verification of the analyte. The method was linear from 2 to 500 ng/mL. The limit of detection was 0.5 ng/mL, and the intraday precision and accuracy of the method were better than 25% at the LLOQ and across the linear range (n=18). The method was applied to the determination of cis- and trans-permethrin following oral dosing in corn oil. The method was able to quantify cis- and trans-permethrin in plasma up to 24 hours following dosing. Supported by the Council for Pyrethroid Human Risk Assessment (CAPHRA).

2898 Acute Water Column Effects Concentrations (LC50, LC90) for Three Commonly Used Insecticides, Two Neonicotinoids (*Acetamiprid* and *Imidacloprid*), and a Recently Registered Phenylpyrazole (*Fipronil*), Exposed to Common Commercially Cultured Shrimp (*Marsupenaeus japonicus*)

M. A. Ghorab[4], M. J. Zwiernik[3], H. Omar[2], H. Samir[4] and M. S. Khalil[1]. [1]*Central Agricultural Pesticides Laboratory, Agriculture Research Center, Alexandria, Egypt;* [2]*Marine Invertebrates Laboratory, NIOF, Alexandria, Egypt;* [3]*Michigan State University, E. Lansing, MI and* [4]*Environmental Toxicology Laboratory, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt.*

In the current study the toxicity impact of some insecticides namely acetamiprid, imidacloprid and fipronil were investigated on shrimp (*Penaeus japonicus*), as bio-indicator to pollution of marine water. The recorded data showed that fipronil was the most toxic insecticide during the different intervals 48, 72 and 96 hrs of exposure. The estimated values of LC50 of fipronil were 5.18, 1.29 and 0.56 ppm after exposure by 48, 72 and 96 hrs, respectively. Otherwise, acetamiprid was the less toxic insecticide with 2335.49, 256.79 and 214.33ppm of LC50 during the intervals periods, 48, 72 and 96 hrs, respectively. Imidacloprid showed moderate toxicity to shrimp with 1011.30, 240.81 and 141.42 ppm of LC50 during 48, 72 and 96 hrs of exposure, respectively. On the other hand, data showed that acetamiprid recorded the least mortality after 96 hrs of exposure by 66.67% with the highest concentration, followed by fipronil and Imidacloprid with 76 and 83.33%, respectively.

2899 Role of the Hepatic Endocannabinoid System In Chlorpyrifos-Induced Lipid Accumulation In McArdle-RH7777 Hepatoma Cells

S. Kondakala, C. Mulligan, J.-H. Lee, M. Ross and G. E. Howell. *College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.*

One of the major hepatic ramifications of type 2 diabetes is the occurrence of nonalcoholic fatty liver disease or hepatic steatosis which is a condition where triglycerides and other lipids accumulate within the hepatocyte. Given recent studies indicating a role of environmental exposures in the pathogenesis of type 2 diabetes, the current study was designed to determine if direct exposure to the most widely used organophosphate insecticide, chlorpyrifos (CPS), could alter hepatocyte lipid metabolism to promote neutral lipid accumulation. To determine if CPS exposure increased intracellular lipid accumulation, McA-RH7777 cells were incubated with CPS for 48 hours then lipid accumulation was determined by Oil Red O staining and intracellular triglyceride levels. Exposure to CPS significantly increased Oil Red O staining in a concentration-dependent manner. This increase in Oil Red O was paralleled by an increase in intracellular triglyceride levels following CPS exposure for 48 hours. Although inhibition of acetylcholinesterase is the primary route of CPS toxicity, recent studies have demonstrated CPS exposure can have non-cholinergic toxicities including alteration of the endocannabinoid system. The effect of CPS exposure on the endocannabinoid system was explored as a possible mediator of CPS-induced lipid accumulation. McA cells were exposed to CPS for 8 and 24 hours then the 2-AG and AEA levels in the treatment media and cell lysates were determined. Exposure to CPS significantly increased media levels of 2-AG at 8 and 24 hours and lysate levels of 2-AG at 8 hours while exposure to CPS for 24 hours resulted in decreased hydrolysis of 2-AG. Thus, these increases in 2-AG appear to be due, at least in part, to decreased 2-AG degradation. Interestingly, the CPS-induced reduction in 2-AG hydrolysis was greater than that produced by JZL-184, a specific monoacylglycerol lipase (MAGL) inhibitor. In conclusion, the present study indicates CPS exposure can have a direct effect on the hepatocyte to produce neutral lipid accumulation which may be mediated, in part, by activation of the hepatic endocannabinoid system. However, additional *in vivo* studies are warranted to determine if our currently observed *in vitro* effect has systemic relevance.

2900 1,3-Dichloropropene: Hazard Identification and Risk Assessment for Occupational and Bystander Inhalation Exposures

A. Rubin, C. Aldous, I. Reeve, P. Lohstroh, S. Rinkus, C. Lewis, M. Wilbur, E. Kwok, S. Koshlukova, S. Beauvais and M. Verder-Carlos. *Dept. of Pesticide Regulation, California EPA, Sacramento, CA.*

The fumigant 1,3-dichloropropene (1,3-D) is injected into soil or applied through drip irrigation to control soil dwelling pests. Pesticidal action is attributed to enzyme inactivation via sulfhydryl or hydroxyl binding. In mammals, 1,3-D is a respiratory irritant and asphyxiant, and is also absorbed through the respiratory tract to generate systemic toxicity. Human exposure results from volatilization and off-site transport. We evaluated the risk for health effects from occupational and bystander inhalation exposure. The acute inhalation benchmark concentration (BMCL1σ) used to address short-term risk was 49 ppm for bodyweight decrements in rats on day 3 in a 13-week inhalation study. Regional gas dose ratio (RGDR) scaling was used to calculate human equivalent concentrations (HEC) for occupational (oc) and non-occupational (noc) exposures (HECoc/noc=11/33 ppm). The subchronic NOEL was 10 ppm (HECoc/noc=0.19/0.56 ppm) based on nasal epithelial hyperplasia in rats in a 13-wk study. The chronic NOEL was 5 ppm (HECoc/noc=0.16/0.49 ppm) based on nasal epithelial hyperplasia and urinary bladder pathology in mice in a 2-yr study. Oncogenic potency was estimated using incidence of bronchioloalveolar adenomas in mice from a 2-yr study. BMC analysis was combined with evidence of genotoxicity to generate potency factors expressed as air units of concentration (AUCoc/noc=0.018/0.0059 ppm-1). Exposure estimates for agricultural workers and bystanders were calculated using 1,3-D specific and surrogate data and probability-based models for short-term, seasonal, annual and lifetime scenarios. Short-term breathing zone air concentrations for workers were 0.04-38 ppm. Lifetime breathing zone air concentrations were 0.001-2.2 ppm for workers and 0.069-0.670 ppb for bystanders. HECs, AUCs and exposure values were combined to generate risk estimates that were compared to target values; estimates exceeding targets included occupational and non-occupational scenarios for all exposure lengths.

2901 Social Determinants in Household Pesticide Exposure: Development of a Conceptual Underlying Model of Risk Perception in Argentinean Families

P. Kandel Gambarte[1], N. Pereiro[6], M. G. Rovedatti[3], S. Aragone[6], A. Ferloni[6], M. G. Cruz[6], F. A. Vidal[6], M. J. Wolansky[2] and B. S. Figar[6]. *[1]ARG Ministry of Health/Hospital Italiano de Buenos Aires (HIBA)/University of Buenos Aires (UBA), Buenos Aires, Argentina; [2]Biological Chemistry, ARG NRC/UBA, Buenos Aires, Argentina; [3]Biological Chemistry/Biodiversity and Experimental Biology, UBA, Buenos Aires, Argentina; [4]Epidemiologia, HIBA, Buenos Aires, Argentina; [5]Epidemiologia - Salud Poblacional, HIBA, Buenos Aires, Argentina and [6]Toxicologia, HIBA, Buenos Aires, Argentina.*

This study aims to understand knowledge, attitude and behaviors on home pest control to develop a conceptual underlying model of household pesticide risk perception. A qualitative content analysis was applied on interviews to families having little children belonging to middle class from a metropolitan Hospital at Buenos Aires. Ten families were interviewed, taped and transcribed verbatim. Horizontal and vertical analyses were used to identify principal domains and divergence between the narratives of participant´ profiles. Seven families lived in apartment buildings; in 6 interviews both parents were present. Their average age was 34.5 (SD 3.57), and 12/20 (60.0% - CI95% 36.4-80.0) had high school education level or more, and 1.2 (SD 0.42) children. In this initial phase of the study we found a number of misbeliefs, attitudes and behaviors with recognized potential to influence pesticide exposure. Knowledge: Interviewees often failed to properly classify pests, pesticides, disinfectants and cleansing products. Attitude toward household pesticide exposure: Save a few exceptions, parents naturalized the monthly fumigation provided by pest control contractors serving the apartment community. Behaviors: Another common finding was a lack of safety standards during and after application of what parents considered acceptable products for home pest control. Use of pesticide products formulated with active ingredients phased out or not recommended for home pest control were also apparent. These results strongly suggest that in our society there is unawareness about the health risks posed to humans through exposure to pesticides, arguing for educative and regulatory actions to prevent pesticide toxicity in susceptible individuals, i.e. women at child-bearing age and little children. Knowledge categories appear the former to be addressed for effective behavioral change (work in progress).

2902 Pyrethroid Metabolism and Exposure Assessment in Egyptian Agricultural Workers

M. Himmel[3], B. McGarrigle[3], R. Singh[3], A. A. Ismail[1], G. Abdel Rasoul[1], O. Hendy[1], M. Bonner[3], D. Rohlman[2,4], D. Aga[3] and J. Olson[3]. *[1]Menoufia University, Shebin El-Kom, Egypt; [2]Oregon Health & Science University, Portland, OR; [3]State University of New York, Buffalo, NY and [4]University of Iowa, Iowa City, IA.*

Pyrethroid (PYR) insecticides, including lambda-cyhalothrin (λCH) and alpha-cypermethrin (αCM), are used in both agricultural and residential settings in Egypt and other parts of the world. They act by prolonging the open time of sodium channels, increasing the duration of neuronal excitation. PYRs are active in their parent form with metabolism by cytochrome P-450 and carboxylesterase resulting in the formation of inactive metabolites, which serve as biomarkers of exposure. Since little is known about the metabolism of λCH, we developed a sensitive and specific HPLC method for analysis of all major λCH metabolites. While the metabolism of many PYRs produce the metabolite 3-phenoxybenzoic acid (3-PBA), lambda-cyhalothric acid (LC-Acid) is a specific metabolite unique to λCH and cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA) is a specific metabolite for αCM. Pooled human liver microsomes were used to assess kinetic parameters (Km and Vmax) for the metabolism of λCH. The formation of 3-PBA, and LC-Acid have Vmax values of 420 and 468 pmol/min/mg protein, respectively and Km values of 20 and 13 μM λCH, respectively. These values are similar to those reported earlier for αCM. A new liquid chromatography tandem mass spectrometry (LC-MS/MS) method was also developed to measure ng levels of these PYR metabolites in urine, which provides a sensitive and specific biomarker of exposure. This method was used to assess the wide range of exposures to λCH and αCM in Egyptian adolescent agriculture workers that apply these pesticides to the cotton fields for up to 10 consecutive days and age-matched non-applicators. Together, these studies provide data that can be used to examine the impact of PYR exposures on neurobehavioral symptoms and performance. (Supported in part by NIEHS and the Fogarty Institute R21 ES017223 and R01 ES022163, Rohlman).

2903 Effect of Mancozeb and CuCl2 on Metal Deposition and Lipid Peroxidation in the Rat Hippocampus

K. A. McGovern and L. D. Trombetta. *PHS, St. John's University, Queens, NY.*

Rising concern over the neurotoxic properties of pesticides has prompted the toxicological evaluation of these chemicals and their possible impact on neurodegeneration. Mancozeb (MZ) is a widely used ethylene bis-dithiocarbamate (EBDC) fungicide that exhibits low mammalian toxicity. MZ's structure contains the transition metals Mn2+ and Zn2+ and an organic backbone that are all known to cross the blood brain barrier. It was investigated whether doses of MZ or MZ in combination with CuCl2 altered metal deposition in the rat hippocampus resulting in lipid peroxidation. Rats were administered MZ (50 and 100 mg/kg), CuCl2 (5 and 10 mg/kg) or combinations of the two chemicals (50 mg/kg + 5 mg/kg or 100 mg/kg + 10 m/kg) by oral gavage 3 times a week for one month. Zn, Cu, and Mn levels in the hippocampus were quantified by way of inductively coupled plasma mass spectrometry (ICP) whereas lipid peroxidation was assessed using the biomarker malondialdehyde (MDA). All data was analyzed using GraphPad Prism with a $p < 0.05$. Zn levels were quantified as being the most abundant in the hippocampus followed by Cu then Mn. Zn, Cu and Mn levels were found to be highest in rats treated with 100 mg/kg MZ and 10 mg/kg CuCl2. All treatment groups other than the 50 mg/kg MZ showed significant increases in MDA content when compared to non-treated rats. Rats exposed to combinations of both MZ and CuCl2 resulted in increased levels of lipid peroxidation when compared to MZ doses alone. The findings indicate that exposure to these chemicals in combination may lead to increased neurotoxicity greater than either alone. Metal combinations may be involved in neurodegenerative diseases.

2904 Toxicity of Atrazine and Glyphosate Based Formulations on *C. elegans*

L. P. Tejeda-Benitez, M. C. Garcia-Espiñeira, A. Bustamante and J. T. Olivero-Verbel. *School of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia.*

Atrazine and glyphosate are herbicides massively used in agriculture for crop protection, and in the case of glyphosate, it is currently employed for eradication of illegal crops. Upon application, these herbicides are available to the biota in different ecosystems. The aim of this research was to evaluate the toxicity of commercially available products containing glyphosate and atrazine as active compounds. *Caenorhabditis elegans* was used as a biological model, and the nematodes were exposed to 0.01 - 1000 µM of each pesticide, independently or as a mixture, based on the concentration reported for each pesticide in the respective formulation. Lethality, locomotion, growth, and fertility were measured as endpoints. Effects on gene expression were monitored utilizing GFP transgenic strains containing hsp-3, hsp-70, sod-1, sod-4, gpx-4, gpx-6 genes. K medium was utilized as a control. Atrazine caused lethality of 12, 15, and 18% for 10, 100, and 1000 µM, respectively, displaying a dose dependence trend. Glyphosate produced lethality of 20, 50, and 100% at 0.01, 10, and 100 µM, respectively. Locomotion inhibition ranged from 21 to 89% at the lowest and maximum tested concentrations for atrazine; whereas for glyphosate, exposure to 10 µM inhibited 87%. Brood size was decreased by 67% and 93% after treatment to 0.1 and 10 µM atrazine, respectively; and by 23 and 93% after exposure to 0.01 and 10 µM glyphosate, respectively. There were not significant differences in growth between treatments. Changes in gene expression occurred in genes sod-1, sod-4, and gpx-4 with fluorescence expression about twofold higher than control for 100 µM atrazine and 10 µM glyphosate. The effects observed for the mixture of these herbicides were additive for lethality and locomotion; and for fertility, these were slightly synergistic. In short, glyphosate, atrazine, and their mixture induced a wide range of toxic responses on *C. elegans*.

2905 A Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK/PD) Model for Malathion in Rats

K. T. Bogen. *Health Sciences, Exponent, Oakland, CA.*

A physiologically based pharmacokinetic (PBPK)/pharmacodynamic (PD) model for uptake, metabolism and disposition in rats of the widely used and relatively weakly cholinesterase-inhibiting organophosphate (OP) pesticide malathion was developed by adapting malathion previously related models developed for malathion and chlorpyrifos (another OP pesticide) and fitting data from seven unpublished studies funded by the malathion manufacturer as well as from one previously published metabolism study in rats. The model was fit jointly to sets of data on urinary/fecal (UF) metabolite excretion and urinary excretion of malathion primary metabolites (MPM) as well as to 8 additional sets of dose-response (DR) data on RBC AChE inhibition (including male and female rats exposed to malathion doses administered either acutely by gavage at postnatal day 11 (PND11), by gavage for 11 days starting at PND11, or by adult-rat dietary intake for durations of 28 or 90 days). Predictions were also compared to data relating AChE inhibition to time following a single 150-mg/kg dose. The DR data were fit well by the PBPK/PD model as well as by a simple, analytic, Michaelis-Menten (hyperbolic) response function (r = 0.98 for each fit). The data and fits obtained indicate that malathion AChE (PD) effects do not accumulate during multiday or subchronic malathion exposure in rats. The model predicts that 8-hour/day malathion exposures of 10.5 (or 7.7) mg/kg/day induce levels of peak ≤10% RBC AChE inhibition in 25-g rat pups or 250-g adult rats corresponding to ~2.0- (or 1.6-) µmol/L peak blood concentrations of malathion. Such PBPK/PD-based predictions for rats can be compared directly to peak malathion concentrations in blood of humans (e.g., occupationally) exposed to malathion as a basis for mechanistically based interspecies exposure and risk extrapolation for this pesticide.

2906 Fate of Lambda-Cyhalothrin during Tobacco Planting and Processing

L. Chen, H. Cui, J. Cai, L. Pan, X. Wang, M. Fan, Y. Jia, L. Zhao and H. Liu. *Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China.* Sponsor: R. Krieger.

Pesticide residue is one of the most important issues affecting the quality and safety of tobacco. Lambda-cyhalothrin was widely used in tobacco cultivation and was frequently detected. To investigate the key procedure of pesticide residue control, the behavior of lambda-cyhalothrin on tobacco, from treatment to harvest, and its fate in tobacco processing, including flue-curing, threshing and redrying, primary processing, were studied. Residue analysis using GC-MS/MS was performed on 5 samples using matrix-matched standards. Average recoveries of lambda-cyhalothrin in tobacco were in the range of 97 - 100% with relative standard deviations lower than 13% at three spiking levels of 0.05, 0.25 and 0.5 mg/kg. The LOD and LOQ of lambda-cyhalothrin was 0.001 and 0.003 mg/kg at the signal-to-noise ratio (S/N) of 3 and 10, respectively. Field experiments including the residue dynamic experiment and final residue experiment in supervised field trials designed according to the pesticide label recommendations were conducted in Linyi and Qujing during 2014 according to "Guideline on pesticide residue trials" issued by Ministry of Agriculture of the People's Republic of China. At two geographical experimental plots, lambda-cyhalothrin residue in tobacco leaves showed a different dissipation rate, with half-lives of 16.7 days in Linyi and 8.1 days in Qujing, respectively. Residues persistence varied in two field trials, suggesting that it might be affected by some physical and chemical factors like light, heat and moisture, growth dilution factor, soil characteristics. The eliminated percentage of lambda-cyhalothrin in the flue-curing processing is in the range of 9-22%. No effect on the threshing, redrying and primary processing was observed.

2907 Flupirtine Protects Against Permethrin/DEET-Induced Brain Injury in a Rat Model for Gulf War Illnesses

M. B. Abou-Donia, S. Abdelwahab, S. K. Acheson and S. Swartzwelder. *Department of Psychiatry & Behavioral Science, Duke University Medical Center, Durham, NC; Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.* Sponsor: M. B. Abou-Donia.

Of almost 700,000 US military personnel who served in the Gulf War from August 1990 to June 1991 over 300,000 veterans developed symptoms known as the Gulf War veterans' illnesses (GWVI), characterized by persistent pain, fatigue, and cognitive deficits. Flupirtine has been used in the treatment of patients with memory and sensorimotor deficits. In this study, we investigated the effect of flupirtine in protecting from GWVI in our previously developed rat model for the GWVI. Four groups of 20 male Sprague-Dawley rats were treated for 60 days with daily dermal doses of 40 mg/kg DEET and 0.13 mg/kg permethrin in 70% ethanol alone, or with an i.m injection of 10 mg/k flupirtine; the other two groups were treated with flupirtine or the vehicle alone. No visual differences were noted in general condition or body weight between control and treatment groups. DEET/permethrin treatment caused brain neuronal cell death exhibited in the cerebral cortex, the hippocampus and Purkinje cells in the cerebellum. Concurrent treatment of DEET/permethrin and flupirtine greatly protected brain neuronal cells from DEET/permethrin-induced neuronal cell death. Water maze data revealed no substantive effect of DEET/permethrin or flupirtine on traditional measures of spatial acquisition. However, probe trial data suggest that animals treated with DEET/permethrin and vehicle tended to perseverate by searching only in the former goal area. Animals treated with DEET/permethrin and flupirtine concurrently, did not appear to perseverate and widened their search area as the probe trial progressed. The results indicate that concurrent treatment with flupirtine protected the animals fromDEET/permethrin-induced brain neural injury (Supported in part by a DOD Contract Number; W81XWH-09-1-0537).

2908 Age-Dependent Toxicokinetics (TK) of Cis-Permethrin (CIS) in Different Life Stages of Sprague-Dawley (SD) Rats

T. Mortuza, J. Pang, B. S. Cummings, J. V. Bruckner and C. A. White. *Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA.*

Cis-Permethrin (CIS), a class I pyrethroid, has been extensively used in domestic and agricultural settings in US, Canada, and Europe. Nevertheless, there are only limited TK data available for development and validation of a comprehensive physiologically-based Toxicokinetic (PBTK) models for subpopulations, notably children. The purpose of the study was to characterize the age- and dose-dependency of plasma and tissue CIS time-courses. Adult male and two groups of young (15- and 21-day-old) SD rats of mixed gender were administered 3 doses of CIS as a single oral bolus in 5 mL corn oil/kg. The adult rats were given 60, 90, and 120 mg CIS/kg; weanlings (21-day-old) were dosed with 45, 60 and 90 mg CIS/kg; and 15-day-olds were dosed 15, 30, and 45 mg CIS/kg. Serial sacrifices were performed (N=4/time-point) at 1, 2, 4, 6, 8, 12, and 24 hr post dosing for collection of plasma, brain, liver, muscle, and fat for analysis by HPLC. CIS was absorbed more rapidly in the pups, as their plasma, brain and liver CIS levels were much higher than in adults for the first 2-4 hr post dosing. Thereafter, CIS was eliminated more rapidly from the plasma and tissues of the pups, such that pup AUCs were only modestly higher than corresponding adult values. Brain CIS levels di-

minished more slowly than plasma levels in both age groups, remaining relatively constant between 12 and 24 hr. In contrast, CIS levels rose progressively in fat during the 24-hr monitoring period, achieving very high concentrations in adults. Preweanlings and weanlings, however, had no visible fat. The lack of this major storage depot may be an important contributor to the age-dependent difference seen in CIS TK. Supported by the Council for Advancement of Pyrethroid Human Risk Assessment (CAPHRA)

2909 Extended One Generation Reproduction Study of Amitraz

G. Burin. *Technology Sciences Group Inc., Washington, DC.*

Amitraz is an acaricide and insecticide used on animals and in apiaries. Mammalian toxicity is based on alpha adrenergic agonist activity. Following the general approach described in OECD Guideline 443, an extended one generation reproduction study of amitraz was recently completed in Sprague-Dawley rats using the gavage route of dosing. Animals were administered 0, 0.5, 1.5 or 7.5 mg/kg of technical grade amitraz for nine weeks and then mated. Dose levels were selected based on a range-finding study showing mortality in parents and pups at higher dose levels. Immunotoxicity screening was not included in this study, but was assessed in a separate study. Filial animals were assigned to four cohorts (1A, 1B, 2A, 2B) for targeted testing and received amitraz by gavage starting on Day 7, while the parental generation dams continued to be dosed until weaning of the F1 generation. Cohorts 1A, 1B and2A were dosed for at least 70 days postweaning. Cohort 1A was examined for effects on reproductive tissues and general toxicity after potential gestation, lactation and direct dosing for approximately 90 days. Cohort 1B was retained for the potential parenting of a second generation. Cohort 2A was assessed for auditory startle, functional observation battery and neuropathology after approximately 7 weeks of direct exposure and Cohort 2B was assessed for neuropathology on Post Natal Day 22. All cohorts were examined for sexual maturation, the presence of nipples in male pups and anogenital distance. Examination of reproductive parameters indicated that there was no evidence of treatment-related effects on the OECD criteria that would trigger the need for a second generation. The only apparent effect on parental and filial animals was a decrease in body weight observed primarily in the high dose group. 1Charles River Laboratory, Horsham PA; 2Technology Sciences Group Inc., Washington DC; 3Merial- A Sanolfi Company, Duluth GA; 4Virbac Animal Health, Ft. Worth TX; 5Veto-Pharma, Villelebon-sur-Yvette France.

2910 Chronic Kidney Disease of Unknown Etiology (CKDu): Association with Blood Level of Organochlorine Pesticides and Associated Mechanism

R. Ghosh[2], N. Singh[2], V. Tyagi[2], P. Kare[2], B. D. Banerjee[2], O. P. Kalra[2] and A. K. Tripathi[2]. [1]*Biochemistry, University College of Medical Sciences and GTB Hospital (University of Delhi), Delhi, India and* [2]*Medicine, University College of Medical Sciences and GTB Hospital (University of Delhi), Delhi, India.*

Chronic kidney disease of unknown etiology (CKDu) is a subset of chronic kidney disease (CKD) without any known etiology and may be associated with agrochemical exposure as evidenced by several epidemiological studies from Mexico, Central America and number of Asian countries including India. Organochlorine pesticides(OCPs) are agrochemical which has been detected in the blood of general population. Involvement of OCPs in inducing CKD has not been investigated in depth so far and it is possible that these nephrotoxic chemicals having long half life persist in the environment. Therefore, the present study has been designed to investigate the association of blood level of OCPs with CKDu to establish possible mechanism involved. Two groups of study subjects of 200 in each were recruited for this study. Group 1: Healthy subjects and Group 2: Patients with chronic kidney disease of unknown etiology (CKDu). Blood levels of OCPs of each subject of both the groups were analyzed by gas chromatography. Also, blood cells (PBMC) were treated with prevalent pesticides namely α-HCH, α-endosulphan, p,'p'-DDE and aldrin. These exposed cells were tested for oxidative stress by DCFH-DA method and DNA damage by comet assay. mRNA expression of NADPH oxidase and eNOS were carried out by real time PCR. Increased level of OCPs namely α-HCH, Aldrin, p,'p'-DDE and α-endosulfan were observed in CKDu patients as compared to healthy subjects. The levels of these pesticides correlated significantly with the eGFR of CKDu patients. Logistic regression analysis revealed association of α-HCH (OR-2.52; 95% CI-1.552-4.11), Aldrin (OR-3.07; 95%CI-1.71-2.06) and α-endosulfan (OR-2.99; 95% CI-1.01-8.83) with CKDu on adjustment of age, sex, BMI, total lipid content. α-HCH, Aldrin, p,p'-DDE and α-endosulfan induced superoxide generation, DNA damage in PBMC in a dose dependent manner. These pesticides also found to induce mRNA of NADPH oxidase (p47phox) and eNOS indicating OCP-mediated oxidative stress as a possible mechanism of renal dysfunction observed in CKDu patients.

2911 Perinatal Exposure to Mancozeb Followed by Re-Exposure in Adulthood Alters Neurogenesis and Stress Protein Gene Expression in the Hippocampus of Long-Evans Rats

M. Akhtar and L. Trombetta. *PHS, St. John's University, Queens, NY.*

In 2007, Mancozeb (an ethylenebisdithiocarbamate) was ranked nineteenth among fungicides due to its usage of 6 million pounds to control plant diseases on food and feed crops both in the field and during storage. Mancozeb and its metabolite ethyelenthiourea are known for causing thyroid toxicity, leading to altered thyroid function. Since thyroid hormones are important for proper development of the mammalian nervous system, altered thyroid hormone levels may also affect neural stem cells in the dentate gyrus of the hippocampus. Impairment during neurodevelopment due to Mancozeb exposure may lead to neurodegenerative diseases, especially upon further challenge to environmental stressors during adulthood. This study investigated the ability of Mancozeb to alter the expression of genes involved in neurogenesis and stress-induced proteins in the hippocampi of rats exposed perinatally followed by re-exposure during adulthood. Time-pregnant Long-Evans Dams were exposed to 0, 50, and 100 mg/Kg body weight Mancozeb from gestational day 7 (GD-7) to postnatal day 21 (PND-21). Pups from each group were re-exposed with the same dose as dams from PND-60 to PND-90. At the end of exposure, total thyroxine (T4) blood level in dams and re-exposed animals was significantly decreased. Hippocampi from re-exposed animals were isolated and subjected to real-time PCR analysis for gene expression profiling. Mancozeb exposure significantly up-regulated the expression of genes involved in neurogenesis, such as Nrp1, Sox-2, Sox-8, Egf, and Tgfb-1, and stress protein genes, such as Hspa1a, Hspa1 (Hsp70 super family), and Dnajc1 (Hsp40). Mancozeb's ability to disrupt expression of genes involved in neurogenesis while simultaneously increasing the expression of genes that repair misfolded proteins, indicates Mancozeb's potential to cause developmental neurotoxicity.

2912 Serum Autoantibodies to Neural-Specific Proteins as Objective Biomarkers for Gulf War Illness

M. B. Abou-Donia[3], K. Sullivan[4], L. Conboy[2] and E. Kokkotou[2]. [1]*Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA;* [2]*New England School of Acupuncture, Harvard Medical School, Boston, MA;* [3]*Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC and* [4]*School of Public Health, Boston University, Boston, MA.* Sponsor: M. B. Abou-Donia.

The hallmark of the 1990-1991, Gulf War Veteran's Illnesses (GWI) is neural degeneration that is consistent with symptoms related to nervous system injury and confirmed by experimental studies. We carried out a study to investigate the development of a serum biomarker assay to detect circulating autoantibodies proteins associated with the nervous system in sera of a group of 20 veterans of the Gulf War who reported having symptoms of Gulf War Veteran's Illnesses (GWI) and 10 symptomatic controls with chronic back pain who did not have GWI. A panel of 9 proteins present in axons, dendrites and myelin sheath that are affected by neuronal degeneration were used for the screening by western blotting: neurofilament triplet proteins (NFP), tubulin, microtubule associated tau proteins (tau), microtubule associated protein-2 (MAP-2), myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP), and glial S100B protein. The results show significantly elevated levels of circulating Ig-G-class autoantibodies in the veterans with GWI, compared to symptomatic controls, except for S100B. These results showing evidence of neuronal injury and gliosis in the GW veterans with GWI are consistent with recent reports indicating 25 years after the Gulf war, the health of the veterans who developed GWI is not improving and may be getting worse. It is concluded that these serum circulating autoantibodies may be used as diagnostic biomarkers for GWI if confirmed in larger samples. (Supported in part by DOD Contract No. W81XWH-15-1-0641).

2913 Taurine Ameliorated Impairment in Neuromuscular Coordination in Rats administered with Chlorpyrifos

M. G. Akande[2], M. Shittu[1], C. Uchendu[1] and L. S. Yaqub[1]. [1]*Ahmadu Bello University, Zaria, Nigeria and* [2]*University of Abuja, Abuja, Nigeria.*

The aim of the study was to investigate the effects of taurine on neuromuscular coordination in rats exposed to chlorpyrifos. Taurine (C2H7NO3S) is a non-enzymatic antioxidant that is necessary for normal functioning of skeletal muscles. Chlorpyrifos (O,O-diethyl-3,5,6-trichloro-2-pyridyl-phosphorothionate) is a widely used organophosphate insecticide. Fifty male Wistar rats were randomly assigned into five groups of ten rats each. The groups received the following treatments: distilled water, soya oil (1 ml/kg), taurine (50 mg/kg), chlorpyrifos (4.25 mg/kg, 1/20th of median lethal dose) and taurine+chlorpyrifos. The treatments were administered to the rats once daily by oral gavage for 16 weeks. The inclined plane test was used to assess neuromuscular coordination at week 15. Brain malondialdehyde concentration and the activities of brain acetylcholinesterase and brain antioxidant enzymes (superoxide dismutase and catalase) were evaluated after the completion of the study. There was impaired neuromuscular coordination, increased brain malondialdehyde concentration and decreased activities of brain antioxidant enzymes and acetylcholinesterase in the chlorpyrifos group. Conversely, there was improved neuromuscular coordination, reduction in brain malondialdehyde concentration and enhancement of the activities of brain antioxidant enzymes and acetylcholinesterase in the taurine + chlorpyrifos group. The impairment in neuromuscular coordination in the chlorpyrifos group may be due to reduction in acetylcholinesterase activity and oxidative stress that was manifested as increased brain malondialdehyde concentration and decreased activities of brain antioxidant enzymes. It is posited that taurine ameliorated neuromuscular coordination in the rats through its antioxidant and acetylcholinesterase-restorative properties. Taurine may be used for the improvement of neuromuscular coordination in animals exposed to chlorpyrifos in the environment.

2914 Toxic Effects of Permethrin on Male Rabbit Fertility and Immunity

K. A. Abdou[3], A. E.-A. Khalaf[4], M. A. Abo Norag[2] and N. M. Ibrahim[1]. [1]*Animal Health Research Institute, Dokki, Egypt;* [2]*Animal Health Research Institute, Dokki, Egypt;* [3]*Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine Beni Suef University, Beni Suef, Egypt and* [4]*Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.* Sponsor: A.-R. Kadry.

The goal of the present study was to investigate the effect of permethrin dipping (1ml/L) and antioxidant treatment on body weight, testis weight, biochemical parameters, DNA fragmentation, immunological and histopathological changes in treated mature male rabbits. In brief, rabbits were equally divided into 3 groups (12 rabbits each) after 15 days of acclimatization - Group I (control group), Group II (treated with permethrin 7days before killing), and Group III (taking the anti-oxidants, vitamine E and selenium, for 7days prior to dipping). The preliminary results showed that while there is significant decrease in body weight and testis in permethrin-treated group (Group II), no significant change was found in permethrin-treated group with the pretreatment of the anti-oxidants (Group III). Significant decrease in epididymal sperm concentrations was likewise observed in permethrin-treated rabbits regardless of whether rats treated with anti-oxidants. Different extent of decrease or inhibition in sperm motility (type A, B, and C), sperm abnormalities, and biochemical hormone parameters in blood (e.g., serum testosterone, prostatic acid phosphatase, catalase enzyme, and globulins) was also apparent in permethrin-treated rabbits, particularly the ones without pre-treatment of the antioxidants. The antioxidant pretreatment also shows protective role against the toxic effect of permethrin on reduced glutathione and promoting lipid peroxidation upon 3rd and 4th time of permethrin dipping. Similar findings was observed with respect to the protective effects of anti-oxidant treatment against the genotoxic effect (e.g., abnormal DNA fragmentation), immunotoxicity (e.g., declining antibody titer), and histological abnormalities (e.g., intertubular vasculitis) in response to permethrin exposure. In conclusion, whereas permethrin is highly toxic to mature male rabbits including induced reproductive toxicity, immunotoxicity, genotoxicity, and histopathological alterations, the application of antioxidants -vitamin E and selenium- appear to moderate its toxic effects. The present study provides mechanistic insights into permethrin toxicity.

2915 Dietary Chlorpyrifos Affects Inhibitory Control and Attention in Human APOE Targeted Replacement Mice

F. Peris-Sampedro[2], P. Basaure[2], I. Reverte[2], M. Cabré[3], J. L. Domingo[3] and M. T. Colomina[2]. [1]*Biochemistry, Universitat Rovira i Virgili, Reus, Spain;* [2]*Psychobiology, Universitat Rovira i Virgili, Tarragona, Spain and* [3]*Toxicology, Universitat Rovira i Virgili, Reus, Spain.*

There is a consensus on the contribution of organophosphate (OP) pesticides in a wide range of neurobehavioral disorders. Among the most commonly used, chlorpyrifos (CPF) has been often reported to elicit learning and memory impairments. However, little is known about its effects on executive functioning. Human polymorphisms of the apolipoprotein E (apoE) have been shown to confer different cognitive skills on their carriers, but existing research has focused on deciphering the features of the APOE4 genotype as it is the largest genetic risk factor for Alzheimer's disease. The present study was designed to assess whether the interaction between CPF and the three APOE genetic variants could modulate differently attentional performance, inhibitory control and motivation. Human apoE targeted replacement (TR) adult female mice (apoE2, apoE3 and apoE4) were trained to stably perform the 5-choice serial reaction time task (5-CSRTT). Animals received doses of CPF (3.75 mg/kg body weight/day) through the diet for 4 consecutive weeks. Following exposure, a 4-week CPF-free period was established in order to assess recovery. Despite all individuals acquired the task, apoE2 mice showed an enhanced learning, while apoE4 displayed increased premature and perseverative responding. Strikingly, this lack of inhibitory control was reversed by CPF. Overall, CPF induced protracted impairments in sustained attention and motivation, while it reduced anticipatory responding. ApoE3 mice exhibited delayed attentional disruptions due to motivational factors across the wash-out period. The current findings attest potential links between the APOE4 genotype and the cholinergic system, and provide notable evidence of the emergence of CPF-related attentional and motivational deficits.

2916 The Metabolic Disruptor Role of Chlorpyrifos: Genetic Vulnerability and Hormonal Imbalance

F. Peris-Sampedro[3], P. Basaure[3], M. Cabré[2], T. Neri-Gómez[1], I. Reverte[3], J. L. Domingo[2] and M. T. Colomina[3]. [1]*Universidad Autónoma de México, México DF, Mexico;* [2]*Biochemistry, Universitat Rovira i Virgili, Reus, Spain and* [3]*Psychobiology, Universitat Rovira i Virgili, Tarragona, Spain;* [4]*Toxicology, Universitat Rovira i Virgili, Reus, Spain.*

In recent years, a progressive increase in obesity and type 2 diabetes has been observed in developed countries. Environmental toxicants may disrupt metabolism in a number of ways, including the impairment of both glucose and lipid homeostasis or the alteration of feeding behavior. Multiple signals from gut and fat tissue converge in the brain to control energy balance, where hypothalamus and serotonergic system are key mediators. Following exposure to the pesticide chlorpyrifos (CPF), we recently showed that apolipoprotein E3 (apoE3) mice, which carry the most common isoform of the human apoE, were more prone to become overweighed than apoE2 and apoE4 mice. The current study was aimed at characterizing neuroendocrine and metabolic effects of a subchronic CPF ingestion in apoE3 and C57BL/6N adult male mice. Animals were fed a standard or a CPF-supplemented (2 mg/kg body weight/day) diet for 8 consecutive weeks. Body weight, food intake, lipid and glucose levels, insulin levels and resistance, as well as leptin and ghrelin profiles were determined. Moreover, the expression of the serotonin transporter (SERT) and the 5-HT2A receptor in hypothalamic areas were also assessed. CPF increased food intake, glucose and total cholesterol concentrations, and tended to increase acyl ghrelin levels. However, excess weight gain and increased leptin levels were only attributable to apoE3 mice. On the other hand, the propensity towards a diabetic profile was also notably higher in these animals, who showed a higher homeostatic model assessment index for insulin resistance, as well as higher insulin levels. The expressions of SERT and 5-HT2A receptor were generally increased in CPF-exposed animals, suggesting lower levels of serotonin in hypothalamus. These results support the metabolic-disruptor role of CPF, and further endorse the marked susceptibility of the APOE3 genotype to its effects. These data indicate that both factors may be contributing to the current prevalence of obesity and type 2 diabetes.

2917 Atrazine and Its Metabolites: Aromatase mRNA and *Ex Vivo* Phosphodiesterase Activity

K. D. Yi[2], C. B. Breckenridge[2] and J. W. Simpkins[1]. [1]*Center for Basic & Translational Stroke Research, University of West Virginia, Morgantown, WV and* [2]*Toxicology and Health Sciences, Syngenta Crop Protection, LLC, Greensboro, NC.*

Atrazine (ATR) and its mono-dealkylated (DIA, DEA) but not its dide-alkylated (DACT) or hydroxy (hydroxy ATR, ammeline) metabolites induced aromatase activity in *in vitro* cell lines, which may be mediated via cyclic AMP. We assessed the time- and concentration-dependent effects of ATR and its metabolites on aromatase mRNA expression and inhibition of phosphodiesterase (PDE) activity. Exposure to 10 µM ATR for 2 hr induced ~2-fold increase in aromatase mRNA. Four to 24 hr exposure resulted in ~3-fold increase, and following 72 hr, mRNA levels were 2-fold higher than baseline. A transient increase in aromatase mRNA following 24 hr exposure to 1 µM ATR was observed. ATR metabolites induced modest responses at the highest concentration. Ammeline and hydroxyATR were largely without effects. DACT also had no effects. In *ex vivo*, ATR inhibited PDE activity at concentrations near or above its solubility limits with an IC50 of 39.2 µM. ATR was less effective than 3-isobutyl-1-methylxanthine, a broad PDE inhibitor with an IC50 of 1.75 µM. Metabolites of ATR did not inhibit PDE. However, DACT acted as a competitive inhibitor of ATR. Addition of 500 nM DACT shifted the IC50 of ATR from 39.2 µM to 492.3 µM. These data indicate that high concentrations of ATR cause an induction of aromatase mRNA in *in vitro* steroidogenic cell systems via an inhibition of PDE as shown in *ex vivo* assays. Pharmacokinetic data indicate that ATR is rapidly metabolized *in vivo* to DACT. Steroidogenic cell lines typically used to evaluate aromatase activity are not metabolically competent and thus would not represent what would happen in an animal or human. Using a PBPK model for ATR, it was determined that exposure of humans to the maximum contamination level (3 ppb) of ATR in drinking water would result in tissue concentrations of ATR equivalents of ~ 0.2 pM. In rats, 60 mg ATR/kg BW/day for 4 days results in a peak concentration of 1 µM ATR, which had no effect on aromatase activity or expression *in vitro*. ATR and its mono-dealkylated metabolites are rapidly metabolized after ingestion and thus would not persist long enough to have an effect on aromatase activity *in vivo*. Considering both exposure and pharmacokinetics provides a scientifically defensible interpretation of the lack of relevance of high concentration *in vitro* data and a more appropriate *in vitro* to *in vivo* extrapolation.

2918 Organophosphate-Induced Neuroinflammation, with and without Corticosterone Pretreatment, Is Not Due to Acetylcholinesterase Inhibition

A. R. Locker, K. A. Kelly, L. T. Michalovicz, D. B. Miller and J. P. O'Callaghan. *Centers for Disease Control/National Institute of Occupational Safety and Health, Morgantown, WV.*

Organophosphates (OP) are used worldwide in a variety of settings. Irreversible acetylcholinesterase inhibitors (AChEIs) are used as insecticides (chlorpyrifos (CPF)) and nerve agents (sarin, or surrogate diisopropyl fluorophosphate (DFP)). Reversible AChEIs have been used as a prophylactic as protection from nerve agent and pharmacologically for treatment of myasthenia gravis (pyridostigmine bromide (PB)) and glaucoma (physostigmine (PHY)). Exposure to AChEIs increases acetylcholinergic transmission resulting in salivation, lacrimation, urination, defecation (SLUD) symptoms, muscle rigidity, seizures, paralysis of the heart, and death. Lesser known effects of exposure to some AChEIs (e.g., DFP), paradoxically, include increases in levels of proinflammatory cytokines in the brain, effects exacerbated by chronic (4-7 days) pretreatment with the stress hormone, corticosterone (CORT). Here, we examined neuroinflammatory and AChE effects following peripheral exposure to different reversible and irreversible AChEIs [those that do and do not cross the blood brain barrier (BBB)] with and without CORT pretreatment in male C57BL/6J mice. Using qPCR, we found that the CNS acting irreversible compounds (DFP and CPF) and reversible compound physostigmine (PHY) produced altered levels of proinflammatory cytokines compared to saline treated controls, and further, that pretreatment with CORT augments these brain effects. Administration of the non-BBB crossing reversible AChEI, PB, alone, or following CORT pretreatment produces few inflammatory effects in the brain. Furthermore, irreversible (DFP and CPF) and reversible (PHY) CNS-acting AChEIs all produce decreases in AChE activity levels. Similar effects were not seen following PB exposure, consistent with a lack of brain penetrance. The protection against AChE activity loss and augmentation of neuroinflammation following pretreatment with CORT and subsequent exposure to AChEIs suggests that the neuroinflammation is not a product of AChEIs effect on cholinesterase activity in the brain. Instead, neuroinflammation seen following the exposure to CNS acting AChE inhibiting OPs, with and without CORT, likely is a result of organophosphorylation of yet to be identified protein targets in addition to ACHE.

2919 Jatropha Phorbol Ester Enhanced Serum Amylase Activity in Mice, Leading to Weight Loss and Death

Y. Ishihara, M. Nakao and K. Yamauchi. *Department of Public Health, Kurume University, Kurume, Japan.*

Purpose: Jatropha curcas attracts rising attention as a biodiesel feedstock in the world. However, Jatropha contains various toxic components, generating concerns about its health effects. One of toxic components is potential tumor promoter, phorbol esters. Toxicity of Jatropha phorbol esters has not been fully described. In this study, transdermal toxicity of main component of Jatropha phorbol esters was assessed in mice. Experimental procedures: One of the Jatropha phorbol esters, 12-deoxy-16-hydroxyphorbol-4′-[12′,14′-butadienyl]-6′-[16′,18′,20′-nonatrienyl]-bicyclo [3.1.0] hexane-(13-O)-2′-[carboxylate]-(16-O)-3′-[8′-butenoic-10′] ate (DHPB) (2.5 µg), was applied onto skin of mice. As a positive control, 12-O-Tetradecanoylphorbol 13-acetate (PMA (TPA)) (2.5 µg) a known tumor promoter was applied. Transformation activity of test compound was assayed using Bhas42 cells. All mice were sacrificed at 4 weeks from the beginning of the study, and hematological tests and serum biochemical examination were carried out. Results and discussion: 2.5 µg of DHPB led to decreased eating and weight loss during 4 weeks observation. There were no such symptoms in the mice treated with the same dose of PMA. White blood cells and red blood cells were decreased in DHPB group, and cell differentiation test revealed that lymphocytes were also decreased. Serum biochemical examination showed that marked increase in amylase activity and change in several biochemical parameters, but there was no significant change in lipase activity. Conclusions: These results suggested that the acute toxicity of Jatropha phorbol esters characterized by anorexia may be attributable to the increase of serum amylase activity derived from pancreas or salivary gland. This study was supported by JICA/JST, SATREPS (Science and Technology Research Partnership for Sustainable Development), JAPAN.

2920 Chronic Toxicity of Phorbol Ester, Crude Oil, and Biodiesel Fuel from *Jatropha curcas*

K. Yamauchi, M. Nakao, S. Kinoshita and Y. Ishihara. *Department of Public Health, Kurume University, Kurume, Japan.*

Purpose: *Jatropha curcas* attracts rising attention as a biodiesel feedstock in the world. However, Jatropha contains various toxic components, generating concerns about its health effects. One of toxic components is potential tumor promoter, phorbol esters. We previously examined the acute toxicity of Jatropha phorbol ester. However, chronic toxicity has not been fully described. In this study, chronic toxicity of Jatropha crude oil including Jatropha phorbol ester and biodiesel fuel (BDF), which was made from crude oil by alkaline-methanol treatment were evaluated in mice. Experimental Procedures: 12.5–200 mg of Jatropha crude oil or BDF were applied onto skin of mice twice a week after single application of tumor initiator. As a positive control, 12-O-Tetradecanoylphorbol 13-acetate (PMA), a known tumor promoter, was applied. All survived mice were sacrificed at the 60th week of the experiment, and hematological test and serum biochemical examination were carried out. Results: Jatropha crude oil contained phorbol esters differently from BDF which phorbol ester levels were below detection limit. Despite of the absence of phorbol esters, BDF had tumor-promoting activity. LD50 for chronic administration was 2.0 µg/animal/application for PMA, 50 - 100 mg/animal for crude oil, 100 mg/animal for BDF in 60-week observation. Papilloma was developed in all mice of the BDF-200 mg group but all mice died before papilloma was developed in the crude oil-200 mg group, suggesting that acute toxicity of crude oil was stronger than that of BDF. In addition, safety margin of crude oil was suggested to be narrower than that of BDF. Among all survived mice, there were no significant difference of serum biochemical parameters between the BDF and crude oil groups, but were considerable individual variability within each group. Abnormal findings were observed in white blood cell count and its differential cell count in the crude oil and BDF groups. Conclusions: Chronic exposure to Jatropha crude oil and BDF caused development of papilloma in mice as well as PMA, suggesting that Jatropha phorbol ester contained in Jatropha crude oil and unknown component of BDF developed papilloma. Crude oil has stronger acute toxicity and narrower safety margin than those of BDF, suggesting that the strict regulation in handling of both Jatropha products. This study was supported by JICA/JST, SATREPS (Science and Technology Research Partnership for Sustainable Development), JAPAN.

2921 Enzyme-Linked Immunosorbent Assay for Curcin Protein from a Biodiesel Feedstock Plant *Jatropha curcas*

M. Nakao[1], K. Yamauchi[1], H. Zimila[2] and Y. Ishihara[1]. [1]*Department of Public Health, Kurume University, Kurume, Japan and* [2]*Faculty of Science, Eduardo Mondlane University, Maputo, Mozambique.*

[Introduction] *Jatropha curcas* is highly valued for its potential as biodiesel feedstock especially in developing countries. Residual oil cake is nutrient-rich and can be used as an animal feed. However, toxic materials such as phorbol esters, trypsin inhibitor, and a ribosome-inactivating protein (RIP) curcin in Jatropha seed limit the usage of Jatropha. There is no simple assay method for curcin, though the toxicity of curcin has been reported to be much weaker than that of ricin, well-known RIP. In this study, we developed an enzyme-linked immunosorbent assay (ELISA) for measuring curcin for safety evaluation of Jatropha products. [Experimental] Curcin was purified from the kernel of Jatropha seed. Anti-curcin polyclonal antibody was produced in mice immunized with purified curcin to develop curcin-specific ELISA, and evaluated sensitivity and specificity for measuring curcin contents in Jatropha products. [Results and discussion] Curcin was purified to a single band in a SDS gel electrophoresis used as an antigen to raise an antibody for use in Curcin ELISA. Curcin contents were 16.50 - 23.21 mg/kernel in Jatropha seeds harvested in Myanmar, Philippines and Mozambique, while that of Japanese soybean was 0.005 ± 0.006 mg/kernel. Myanmar Jatropha oil cake contained 12.96 ± 3.20 mg curcin/g. The standard curves for both Jatropha kernel and oil cake were similar, suggesting that the method provides both sensitivity and specificity suitable for assessing Curcin in one Jatropha kernel. The results suggest that this method can be used for risk-management against potential poisoning by Jatropha products. This study was supported by JICA/JST, SATREPS (Science and Technology Research Partnership for Sustainable Development), JAPAN.

2922 Abstract Withdrawn by Author.

2923 Uncovering the Genetic Mechanisms of Imidacloprid Resistance in *Leptinotarsa decemlineata* in the Central Sands Region of Wisconsin

J. Clements, S. Schoville, N. Peterson, Q. Lan and R. Groves. *Entomology, University of Wisconsin-Madison, Madison, WI.*

The Colorado potato beetle (*Leptinotarsa decemlineata*) is one of the major agricultural pests found in the Central Sands region of Wisconsin which leads to crop loss and loss of revenue for farmers. In 1995 imidacloprid was introduced as a systemic insecticide to control populations of *L. decemlineata*, but the resistance ratio (an indicator of insecticide resistance) of *L. decemlineata* to imidacloprid has been increasing since 2000, indicating that the beetles are becoming more resistant. Multiple studies from our lab have demonstrated that the lethal dose to kill 50% of *L. decemlineata* (LD50) in different field populations can vary greatly, suggesting that the beetles' loss of susceptibility may result from different modes of resistance. In this study we determined the LD50 values of imidacloprid among different field populations to observe trends in resistance that have developed and changed over time. Using the LD50 values collected from 2008-2011 together with newly collected LD50 values from 2013 and 2014, we have identified field populations that possess a high degree of resistance. We then generated a transcriptome composed of genes from susceptible and resistant populations by extracting RNA from adult beetles and analyzing it with bioinformatics software. We identified genes that are up- and down-regulated between different susceptible and resistant field populations and mined the data for possible mechanisms of resistance. This data revealed a combination of similar and dissimilar up-regulated genes within resistant populations including 8 similar transcripts that encoded for a known mechanism of resistance, such as ABC transporters and cytochrome P450s. We then chose to target up-regulated genes for RNA interference. During this assay we knocked down multiple up-regulated genes, including a cytochrome P450 and a glutathione related protein. By successfully knocking down these genes in resistant populations, we were able to increase susceptibility to imidacloprid.

2924 Spatial Distribution of Organic Contaminants in Three Rivers of Greater London and SE England

J. Wilkinson, P. Hooda, J. Swinden, S. Barton and J. Barker. *Kingston University London, Kingston-upon-Thames, United Kingdom.*

Many pharmaceuticals and personal care products and other emerging contaminants (PPCP/ECs) such as plasticisers and perfluorinated compounds (PFCs) are ecotoxic in aquatic environments. This work presents spatial distribution of PPCP/ECs (via a standardised method) from 50m upstream to 1km downstream of five sewage treatment work (STW) effluent outfalls in three rivers of Greater London and southeast England (n=75). Two rivers were studied from headwaters to first confluence with another river. Thirteen compounds were investigated: pharmaceuticals and recreational drugs (acetaminophen, amphetamine, benzoylecgonine, β-estradiol, diclofenac, methamphetamine), plasticisers (bisphenol-A (BPA), bisphenol-S, hydroxyacetophenone (HAP)) and PFCs (perfluorooctanoic acid, perfluorooctane sulfonate, perfluorononanoic acid, perfluorobutanesulfonic acid). Inputs from five non-STW/diffuse sources were also investigated including street/surface runoff and field drainage. Quantification was achieved using a validated SPE/HPLC-MS method with limits of quantification between 0.75-4.91ng/L and recoveries of 77-114%. Sampling (April to August 2015) coincided with fish spawning season. Eleven of the thirteen compounds were detected with frequencies ranging from <5-100%. Seven main conclusions were drawn from the data: 1) Nearly ubiquitous presence of studied PPCP/ECs, some PFCs and plasticisers found in headwaters; 2) Greatest attenuation occurs from 250-1000m downstream from STW effluents; 3) Contaminants of human origin (pharmaceuticals and recreational drugs) are predominantly introduced via STW effluents; 4) Compounds of non-human origin (plasticisers and PFCs) can be introduced via street/surface runoff at much higher concentrations than STW effluents; 5) Downstream concentrations of compounds not significantly present in STW effluent (typically PFCs) are diluted by effluent discharge; 6) Overall average concentrations typically did not exceed 50ng/L except for BPA (189ng/L), diclofenac (51ng/L) and HAP (65ng/L); 7) Maximum detected concentrations were 2405ng/L BPA (street/surface runoff) and 1422ng/L BPA (STW effluent). PPCP/EC levels were almost exclusively below those commonly associated with ecotoxic and developmental disruption (except BPA in 2 samples). However, possible combination effects are not well-known.

2925 *In Vitro* Fish Hepatic Metabolism: A Ring-Trial to Evaluate Transferability, Intra- and Interlaboratory Reproducibility

H. M. Peterson[3], M. Embry[7], K. Fay[14], M. J. Bernhard[11], I. Bischof[5], J. W. Davis[1], J. Domoradzki[2], M. Halder[4], X. Han[8], J. Hu[1], K. M. Johanning[9], H. Laue[6], D. Nabb[3], J. Nichols[14], C. Schlechtriem[5], H. Segner[13], L. van der Wal[10] and J. Weeks[12]. [1]*DOW Chemical, Midland, MI;* [2]*DOW Corning, Auburn, MI;* [3]*DuPont, Newark, DE;* [4]*European Commission, Joint Research Centre, Ispra, Italy;* [5]*Fraunhofer IME, Schmallenberg, Germany;* [6]*Givaudan Schweiz AG, Dubendorf, Switzerland;* [7]*ILSI HESI, Washington, DC;* [8]*International Flavors and Fragrances, Union Beach, NJ;* [9]*KJ Scientific, Georgetown, TX;* [10]*OECD, Paris, France;* [11]*P&G, Mason, OH;* [12]*SC Johnson, Racine, WI;* [13]*University of Bern, Bern, Switzerland and* [14]*US EPA, Duluth, MN.*

In vitro methods employing cryopreserved trout hepatocytes and liver S9 fractions can be used to estimate whole-body rates of metabolism, which are then used as model inputs to predict chemical bioaccumulation in fish. Predicted levels of accumulation obtained in this manner are closer to measured values than predictions obtained assuming no metabolism, suggesting that these methods can provide critical information as part of a weight-of-evidence approach to bioaccumulation assessment. Regulatory use of these procedures requires, however, that they be evaluated to determine their transferability and their reproducibility within and among laboratories. Building on previous work, a ring-trial was led by the ILSI HESI with the aim to support the development of an OECD Test Guideline for *in vitro* fish hepatic metabolism. Substrate depletion methods were used to determine the rate at which the two *in vitro* test systems metabolize selected test chemicals. The ring-trial involved seven laboratories and assessed six chemicals (pyrene, 4-n-nonylphenol, fenthion, methoxychlor, deltamethrin, and cyclohexyl salicylate). A reference compound (pyrene) was included in all experiments to "benchmark" the study findings. All chemicals were metabolized in a log-linear manner. The data demonstrated 35% or better inter-laboratory CVs for both test systems. Intrinsic clearance rates (gram liver basis) for each compound, calculated for both test systems, generally agreed within a factor of 5. However, there was no pattern which would suggest that one system consistently yields higher rates of clearance. The incorporation of a benchmarking substance was useful for confirming the activity of different lots of material and identifying anomalous results.

2926 Differences in Genotoxicity and DNA Double Strand Break Repair in Whale Cells and Human Cells After Particulate Hexavalent Chromium Exposure

C. L. Browning[1,2] and J. P. Wise[1,2]. [1]*Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY and* [2]*Graduate School of Biomedical Science and Engineering, University of Maine, Orono, ME.* Sponsor: J. P. Wise Sr..

Particulate hexavalent chromium (Cr(VI)) is a common environmental pollutant and a major human health concern. Cr(VI) is a human lung carcinogen that produces DNA damage, such as DNA double strand breaks, and chromosome instability. We have shown prolonged Cr(VI) exposure inhibits homologous recombination (HR) repair, by targeting Rad51 in human lung cells. In addition, the inhibition of HR repair increases Cr(VI)-induced chromosome instability. Interestingly, our previous work showed acute Cr(VI) exposure produces less chromosome instability in North Atlantic right whale cells than human cells. Right whales are an ideal comparison species to humans due to their long life-spans and coastal habitat. In contrast to humans, cancer is rare in right whales and closely related whale species. In this study, we measured the cytotoxic and genotoxic effect of acute and prolonged particulate Cr(VI) exposure in right whale and human lung fibroblasts. We exposed human and right whale cells to zinc chromate for 24-120 h. We found the right whale cells are less sensitive to Cr(VI) than human cells after both 24 and 120 h exposure. Using the chromosome aberration assay, we show Cr(VI) exposure induces approximately 5 times less chromosome damage in right whale cells than human cells following both 24 and 120 h exposures. We then compared the effect of Cr(VI) exposure on the HR repair protein, Rad51, in each cell line. We show a dose-dependent increase in Rad51 foci formation after 24 h Cr(VI) exposure in both species. After 120 h Cr(VI) exposure, Rad51 foci formation is reduced in right whale cells, but not completely ablated as seen in the human cells. These results suggest that HR repair is active in right whale cells after prolonged Cr(VI) exposure, resulting is less chromosome damage and higher cellular proliferation. This work was supported by NIEHS grant ES016893 (J.P.W.).

2927 The Utility of QSARs in Predicting Acute Fish Toxicity of Pesticide Metabolites: A Retrospective Validation Approach

N. Burden[3], S. K. Maynard[4], L. Weltje[1] and J. R. Wheeler[2]. [1]*BASF SE, Limburgerhof, Germany;* [2]*Dow AgroSciences, Abingdon, United Kingdom;* [3]*NC3Rs, London, United Kingdom and* [4]*Syngenta, Bracknell, United Kingdom.*

Under EC Regulation 1107/2009 there is a requirement for registrants to establish whether pesticide metabolites are potentially harmful to the environment, and as such fish acute toxicity assessments may be carried out. The number of metabolites can be considerable; thus this area of testing may use many vertebrates. EFSA's recent "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" outlines opportunities to apply non-testing methods such as Quantitative Structure Activity Relationship (QSAR) models. Provision of a scientific evidence base to support the use of QSARs in the prediction of the acute fish toxicity of pesticide metabolites, and subsequent regulatory acceptance, could reduce the numbers of animals used. This work examines the potential for QSARs to be used for pesticide metabolites through a retrospective validation approach. Ecotoxicity data for 679 metabolites contained within the Pesticide Properties Database (http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm) were extracted and filtered to restrict the dataset to metabolites with experimental fish LC50 values. QSAR calculations were performed for 190 metabolites using the US EPA's ECOSAR software. The most conservative LC50 value generated by ECOSAR was selected for comparison to experimental LC50 values. The relationship between the predicted and experimental values was assessed. Preliminary analysis revealed a significant correlation between predicted and experimental fish LC50 values ($r = 0.60$, $p < 0.001$, Spearman correlation). For the majority of the metabolites the predicted values were equal to or lower than their experimental values. For the remaining values the mean ratio of experimental:predicted LC50 was 0.24 (SEM 0.03), i.e. QSAR predicted values were on average only 4.1 times higher than those derived experimentally. Considering normal experimental variability, and the risk assessment procedure, these estimates may have regulatory utility. This initial conservative analysis indicates that there is value in further refining the QSAR approach to improve the prediction of acute fish toxicity of pesticide metabolites. Refinements to this analysis will be undertaken before this method can be considered for incorporation into regulatory guidance.

2928 Application of the High Throughput Attagene Factorial Platform to Environmental Monitoring: Characterizing Complex, Environmental Mixtures

B. R. Blackwell[3], A. Schroeder[4], K. Houck[2], G. Ankley[1] and D. Villeneuve[1]. [1]*Mid-Continent Ecology Division, US EPA, Duluth, MN;* [2]*NCCT, US EPA, Research Triangle Park, NC;* [3]*US EPA, ORISE/US EPA, Duluth, MN and* [4]*Water Resources Center, University of Minnesota, St. Paul, MN.* Sponsor: J. Nichols.

Bioassays can be employed to evaluate the integrated effects of complex mixtures of both known and unidentified contaminants present in environmental samples. However, such methods have typically focused on one or a few bioactivities despite the fact that the chemicals in a mixture may exhibit a wide range of activities. High throughput toxicology approaches that can rapidly screen samples for a broad diversity of biological activities offer a means to provide a more comprehensive characterization. To test this concept, twenty-four ambient water samples were collected, extracted, and screened for their ability to interact with or modulate over 80 different transcription factors using the Attagene Factorial™ platform utilized by the US EPA's ToxCast Program. Samples evaluated included 10 water samples collected in varying proximity to a wastewater discharge into the St. Louis River, MN; water collected at five sites along a gradient centered on a wastewater discharge into the Maumee River, Ohio, USA; and eight samples collected in association with a nation-wide USGS surface streams study. For samples collected along the St. Louis River, the greatest number of biological activities were observed at locations closest to wastewater discharge with up to 13 endpoints responding. The Maumee River showed a gradient response in the number of observed activities, ranging from three positive responses observed far upstream of a wastewater discharge to ten positive responses at the mixing zone. TGFb signaling and the aryl hydrocarbon receptor (AhR) activation were the biological activities observed at the lowest sample concentration. For the USGS streams study sites, the number of positive responses ranged from 2 for a sample collected from the Iowa River, IA to 15 for a sample collected from the South Platte River, CO. Estrogen response elements and the AhR were activated at the lowest sample concentration. The results highlight the applicability of high throughput toxicology to screening level assessments of environmental sites. The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.

2929 Microalgae as Biological Model to Assess the Toxicity of Cartagena Bay Water

M. R. Alvear-Alayon[2], M. C. Garcia-Espiñeira[2] and L. P. Tejeda-Benitez[2]. [1]*School of Engineering, University of Cartagena, Cartagena, Colombia and* [2]*School of Medicine, University of Cartagena, Cartagena, Colombia.*

Background: Cartagena Bay is located in the Colombian north western Caribbean, in the most important tourist city in the country. In the western part of the bay, Mamonal Industrial Zone is located, being the most important one in the Atlantic Coast on industrial and manufacturing development. However, it is also the greater contributor of pollutants and wastes to the ecosystem, and any toxicological study has not been done so far. The toxicity of water can be estimated by physicochemical or biological methods. Microalgae are useful organisms for estimating the toxicity levels of surface water. They suffer qualitative and quantitative changes in their population, becoming important biological models in environmental monitoring. Methods: In this study the microalgae Thalassiosira weissflogii was used as a biological indicator for assessing the toxicology of the Cartagena Bay water (Mamonal Zone). Water samples were taken at 10 points along the shore of the Cartagena Bay (Mamonal Zone). Heavy metals were determined and toxicity was evaluated by exposure of T. weissflogii to different water sample solutions by measuring cell density, growth rate and chlorophyll production as endpoints. Results: Cell density and chlorophyll production exhibited toxic effects in surface water of the Cartagena Bay, Mamonal Zone. Sites located near petrochemical industries and the mouth of the Canal del Dique, affluent of the Magdalena River, were particularly the most toxic samples evaluated with inhibition of 90% in cell density, 40% in cell division rate, and 87% in chlorophyll production. Furthermore, the toxic effects evaluated correlated strongly with the concentration of lead in water. Conclusion: T. weissflogii is a sensitive test-organism for seawater toxicity.

2930 Liquid or Chewable: Pharmaceuticals and Personal Care Products' Effects on Insects with Two Different Feeding Methods?

M. J. Pennington and J. T. Trumble. *Environmental Toxicology, University of California, Riverside, CA.* Sponsor: D. Eastmond.

The increasing demand for fresh water has many countries looking to reclaimed wastewater for agricultural purposes. This water usually contains pharmaceuticals and personal care products (PPCPs) that remain after it passes through wastewater treatment plants. We studied the effects of PPCPs at environmentally relevant concentrations on two insects of agricultural significance. Leaf-consuming caterpillars (*Trichoplusia ni*) were fed artificial diet containing PPCPs and reared on tomato plants grown in a semi-hydroponic system containing PPCPs; data regarding growth, development and mortality were taken daily. Phloem feeding aphids *Myzus persicae* were examined on tomato leaf cuttings maintained in water containing PPCPs and allowed to reproduce asexually; population growth data were taken daily over two weeks. PPCPs were found to influence growth rate in aphids. PPCPs, including antibiotics, hormones, and a mix of PPCPs significantly increase mortality and hindered the development of *T. ni*. These results have important implications for the use of municipal wastewater in agricultural ecosystems and possible implications for pest management systems.

2931 Potential Influence of Neonicotinoids on Honeybee Fertility

J. A. Jenkins[2], R. O. Draugelis-Dale[2] and B. K. Hopkins[1]. [1]*Entomology, Washington State University, Pullman, WA and* [2]*WARC, US Geological Survey, Lafayette, LA.*

Neonicotinoids (NEO) are the largest growing class of insecticides globally and have been implicated in honey bee decline and queen failures. Systemic pesticides infiltrate plant pollen and nectar; NEO primarily targets receptor-mediated neurotransmission. The goal of this study was to investigate drone sperm quality after 4 days of trophallactic NEO transference at ecologically relevant concentrations of thiamethoxam (THX) or clothianidin (CLO). Three nucleus colonies were established with a common pool of young worker bees. A cage with male drone honey bees was placed in each of the three nucleus colonies. The drone cages facilitated the worker-feeding of drones, while containing them within the hive. The control colonies were fed a 1:1 (w/v) sucrose/water solution, the second and third colonies were fed low and high concentrations of thiamethoxam (as Platinum 75 SG; 63 ppb and 188 ppb a.i.) or clothianidin (as Belay; (1.8 ppb and 5.5 ppb a.i.) in sugar solutions. Semen was collected and shipped overnight for analysis of sperm motility, intracellular esterase activity, and viability. Acrosomal glycoproteins and nicotinic acetylcholine receptors (nAChr) were detected by lectin- and α-bungarotoxin-binding, respectively. Qualitative analyses of motility indicated higher motilities with the lower NEO concentrations, with each treatment dramatically less motile than freshly collected sperm. Viability data were arcsine (square root) transformed, where both the control and low concentrations of THX- and CLO-exposed bees showed higher viability than the high concentrations ($P = 0.0008$), with no difference between THX and CLO. Within the high NEO concentrations, esterase levels were greater with CLO treatment versus THX ($P<0.0001$), indicating lower sperm quality using THX. This is the first study indicating differential affects of NEO and the presence of nAChr on sperm of honey bees, with implications regarding fertility.

2932 Mutagenic Potentials of Paraquat in the African Catfish, *Clarias gariepinus*

I. Ayanda[2], S. Oniye[1], J. Auta[1] and V. Ajibola[1]. [1]*Ahmadu Bello University, Zaria, Nigeria and* [2]*Covenant University, Ota, Nigeria.* Sponsor: B. Ogunlana.

Direct and indirect contamination of the aquatic environment by pesticides results in a decrease in fish productivity and may elevate concentrations of undesirable chemicals in edible fish tissues. The effects of short-term exposure of *Clarias gariepinus* juveniles to acute concentrations of paraquat, a commonly used herbicide was investigated. Fish juveniles were were randomly divided into six group and exposed to varying concentrations of the herbicide: 0.012, 0.037, 0.055, 0.110, 0.220 and 0.00 mg/l for four days. Fish blood and liver samples were taken after the fourth day and analysed for different erythrocyte abnormalities and damage to DNA using micronucleus (MN) test and comet assay respectively. Liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were tested for using commercial kits. There was a concentration-dependent increase ($p<0.05$) in % tail length of DNA and frequency of erythrocyte abnormalities. The liver enzymes, ALT and AST activities also significantly increased with increasing concentrations of the herbicide. This indicates that paraquat induced DNA damage in *Clarias gariepinus* while also triggering different erythrocyte abnormalities in fish blood. It is important to exercise caution in the application of this herbicide especially in the aquatic environment.

2933 Effects of Norethindrone and Metabolite Ethynylestradiol on Reproductive Parameters in a Marine Fish

L. J. Mills[2], S. Jayaraman[2], G. E. Zaroogian[2], D. Borsay Horowitz[2], T. K. Johnston[1] and S. C. Laws[3]. [1]*Atlantic Ecology Division, ORISE Fellow, US EPA, ORD, NHEERL, Narragansett, RI;* [2]*Atlantic Ecology Division, US EPA, ORD, NHEERL, Narragansett, RI and* [3]*Toxicity Assessment Division, US EPA, ORD, NHEERL, Research Triangle Park, NC.*

Norethindrone (NOR) is a progestin used in human contraceptives and has been detected in low concentrations (ng/L) in aquatic environments. Laboratory experiments were conducted with the marine fish cunner (*Tautogolabrus adspersus*) to evaluate whether NOR could affect reproductive success. Egg production, viability and fertility were assessed daily in spawning cunner treated with nominal concentrations of 0, 0.075 or 0.75 mg/kg NOR by oral gavage on days 0, 4, 8, 12 and 16 of this reproductive experiment. Fish were sacrificed on day 17 and gonadosomatic index (GSI) was determined. Brains and ovaries from all fish were flash-frozen for later determination of aromatase activity (i.e., the enzyme that converts androgens to estrogens). In both NOR treatments, egg production per gram female was significantly reduced relative to controls, while GSI was significantly reduced in males and females from the 0.75 mg/kg treatment. Female mortality in 0.75 mg/kg treatment was more than twice that in controls, suggesting an increase in male aggression. Relative to controls, NOR treatment did not affect aromatase activity in female brains, but significantly decreased aromatase activity in ovaries and male brains. During a separate time-course exposure, plasma from NOR-treated cunner was found to contain both NOR and ethynylestradiol (EE2). Thirty minutes after a single gavage with 7.5 mg/kg NOR, plasma concentrations of NOR averaged 370 ng/ml, while EE2 averaged 18.7 ng/ml. By 24 hours after gavage, the average plasma concentration of NOR dropped to 2.7 ng/ml, while EE2 was 9.7 ng/ml. The pattern of EE2 measured in fish plasma over time suggests enterohepatic recirculation of EE2 is occurring in NOR-exposed cunner. In further support of this concept, only EE2 was found in plasma of NOR-treated fish from the 17-day reproductive experiment. Results indicate the metabolite EE2 is eliminated more slowly than NOR and may pose the greater risk to reproductive success in this marine fish. This abstract does not reflect US EPA policy.

2934 Genome-Wide Transcriptional Responses to Deepwater Horizon Oil in Mahi-Mahi (*Coryhaena hippurus*) Embryos

G. Xu[2], E. Mager[3], M. Grosell[3], E. Hazard[1], S. Courtney[1], G. Hardiman[1] and D. Schlenk[2]. [1]*Medical University of South Carolina, Charleston, SC;* [2]*University of California, Riverside, Riverside, CA and* [3]*University of Miami, Miami, FL.* Sponsor: D. Schlenk.

The Deepwater Horizon (DH) oil spill contaminated the spawning habitats for pelagic fish. Exposure to water accommodated fraction (WAF) of oil from the spill is known to result in cardiotoxicity across fish species. The syndrome is consistently characterized by defects in heart formation and function. Unraveling the potentially diverse molecular mechanism of cardiotoxicity is essential for understanding the hazard posed by complex DH oil mixtures present in the environment. We analyzed the time-course (24, 48 and 96 hpf) transcriptional responses to field-collected DH oil, prepared as a WAF exposure in Mahi-Mahi (Coryhaena hippurus) embryos. The mRNA expression was quantified using high-throughput Illumina RNA sequencing (RNA-Seq) on a HiSeq2500. To analyze the Mahi-Mahi RNA sequencing data, a data analysis pipeline was developed. Reads were mapped to reference transcriptomes using the Diamond Basic Local Alignment Search Tool (Diamond Blast) and subsequently counted using a custom perl script, and subsequent translation of the RNA ID's to gene ID's was performed using the NCBI database. RNAseq was analyzed using DEseq2. Significantly up-/down-regulated mRNAs were identified, and the selected targets were verified by quantitative real-time PCR (qRT-PCR). Downstream gene ontology enrichment analysis was performed using the DAVID Bioinformatics Resources, and network analysis and biomarker identification performed using Ingenuity Pathway Analysis software (Ingenuity Systems, Inc.). Hypothesized transcriptomic responses include AHR-mediated response, HIF1 signaling, immune signaling, Ca2+-cycling and cardiac-as-

sociated genes. This RNA-Seq experiment was successfully applied to a non-model wild, and ecologically significant organism affected by the DH oil spill, and may reveal the molecular mechanisms of cardiotoxicity of DH oil mixtures (Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER)).

2935 Hepatocellular Lesions and Regeneration of the Liver Associated with Microcystin-LR Exposure in Tilapia, *Oreochromis* Sp

S. A. James-Yi[2], L. H. Khoo[4], B. S. Jortner[2], L. A. Murphy[3], D. D. Kuhn[2], D. P. Taylor[2] and S. A. Smith[2]. [1]*Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA;* [2]*Food Sciences and Technology, Virginia Tech, Blacksburg, VA;* [3]*PADLS New Bolton Center Toxicology Laboratory, University of Pennsylvania, Kennett Square, PA and* [4]*Thad Cochran National Warmwater Aquaculture, Mississippi State University, Stoneville, MS.*

Microcystins are cyclic heptapeptide, hepatotoxins released into the water by lysis of cyanobacteria. They have world-wide distribution, populating nearly 100% of bodies of water in some geographical regions. They are of public health concern due to exposure of people and animals (vertebrate and invertebrate) to toxin through drinking water and their association as possible carcinogens at chronic, low levels of exposure. Hepatotoxicity occurs due to inhibition of phosphatases causing cytoskeletal disorganization; organelle toxicity; and nuclear alterations. Fish exposed to cyanobacteria and/or their toxins can exhibit significant morbidity and mortality. Tilapia (*Oreochromis* sp.) were chosen because they are one of the primary fish used in aquaculture and do not attain the same levels of mortality compared to other species of fish. In the present study, juvenile hybrid tilapia (n=28) were intraperitoneally exposed to 700 µg/kg microcystin-LR (MC-LR). Whole blood and liver tissues were collected from a subset of control (IP injection of vehicle only) and experimental populations on Days 1, 3, 5, 7, and 10 post-exposure. Hepatic lesions were assessed using light microscopic examination of toluidine blue-stained sections of epoxy resin embedded tissue (n=1-3/interval), a method which provides enhanced definition and resolution by light microscopy. On Day 1 there was cell swelling and individualization of hepatocytes, along with increases in small, clear, cytoplasmic vacuoles in all animals. By Day 3 the vacuolation was markedly enhanced, largely replacing the cytoplasm of affected hepatocytes. This led to regional necrosis and lobular collapse by Day 5. On Day 7, 1/3 fish continued to have such lesions, but repair and regeneration was prominent in 2/3. Significant regeneration of hepatocytes was noted on Day 10. This study demonstrated that tilapia not only survive hepatotoxic doses of microcystin-LR but also assessed the duration and characterization of effects leading to hepatic regeneration.

2936 Mitochondrial Electron Transport System Function Is Reduced in Salmon Exposed to Contaminants of Emerging Concern

A. Yeh[1], D. J. Marcinek[1], J. P. Meador[3] and E. P. Gallagher[1]. [1]*Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA;* [2]*Department of Radiology, University of Washington, Seattle, WA and* [3]*Northwest Fisheries Science Center, NOAA Fisheries, Environmental and Fisheries Sciences Division, Seattle, WA.*

We have observed the bioaccumulation of a number of contaminants of emerging concern (CEC) in Chinook salmon from certain estuaries of the Puget Sound, WA. Several of these compounds are known inhibitors of electron transport system (ETS) function in mammals. To elucidate the impact of CEC on hepatic ETS function, we used high-resolution respirometry to assess mitochondrial respiratory function in liver of juvenile Chinook after a 30-day dietary exposure to a CEC mixture representative of those measured in field-sampled salmon, including 13 pharmaceuticals and personal care products (PPCPs) and 3 perfluorinated compounds. Exposure groups included 0.1x, 1x, and 10x doses of the model mixture of CEC, based on the CEC analyte concentrations measured in wild fish. Multi-analyte HPLC/MS/MS analysis of Chinook whole-bodies determined bioaccumulation potential for the PPCPs amitriptyline, amlodipine, azithromycin, diltiazem, diphenhydramine, fluoxetine, gemfibrozil, metformin, miconazole, sertraline, triclocarban, and the perfluorinated compounds perfluorodecanoic acid (PFDA), perfluorooctane sulfonate (PFOS), and perfluorooctane sulfonamide (PFOSA). Exposure to the model mixture of CEC increased the rates of mitochondrial State 4 respiration (i.e. leak respiration) by 40-70% relative to controls, but had no effect on either State 3 respiration or uncoupled respiration. As a result, the efficiency of oxidative phosphorylation as measured by respiratory control ratios was 29-42% lower in CEC-exposed fish relative to controls. The observed changes in mitochondrial ETS function were not due to the effects of CEC on liver mitochondrial content, as confirmed by analysis of citrate synthase activities. Our results demonstrate that exposure to environmentally-relevant mixtures of CEC decreases liver mitochondrial function in Chinook salmon, and support use of analyzing mitochondrial function as a novel biomarker of sublethal effects of CEC exposures in wild fish. Supported by WA Department of Ecology G1300089, NIEHS Superfund (ES-04696), WA Sea Grant (R/OCEH-5), and NIH RC2 AG036606.

2937 Exploring the Effects of Temperature and Resource Limitation on Mercury Bioaccumulation in *Fundulus heteroclitus* Using Dynamic Energy Budget Modeling

B. W. Clark[3], K. Buckman[2], D. Miller[4], A. Bertrand[3], I. Kirby[3], D. Champlin[3], C. Chen[2] and D. Nacci[3]. [1]*Dartmouth College, Hanover, NH;* [2]*Atlantic Ecology Division, Dartmouth College, Hanover, NH;* [3]*Atlantic Ecology Division, US EPA, Narragansett, RI and* [4]*Mid-Continent Ecology Division, US EPA, Grosse Ile, MI.* Sponsor: L. Mills.

Dynamic energy budget (DEB) theory provides a generalizable and broadly applicable framework to connect sublethal toxic effects on individuals to changes in population survival and growth. To explore this approach, we conducted growth and bioaccumulation studies that contribute to a DEB model, which allows joint acquisition and interpretation of chemical exposure and stressor effect information that readily translates into demographic rate changes. Ultimately, we will develop a test framework that connects molecular mechanistic information to population level fitness. As a test case, we are studying the effects of temperature and resource availability on mercury (Hg) accumulation in the estuarine fish *Fundulus heteroclitus*. Methylmercury (MeHg), a potent neurotoxin and global contaminant, accumulates in marine food webs raising concerns for human exposure. Warming temperatures, occurring with global climate change, may increase MeHg production and bioaccumulation through increased food consumption and MeHg accumulation. However, higher temperatures may also lead to increased growth and reduced MeHg accumulation through somatic growth dilution. In the current study, groups of juvenile mummichog were fed tuna naturally contaminated with Hg at one of two feeding rates (3.3% or 10% dry body weight/day) while held at either 15 or 27 °C for 28 days. Growth was low in most treatments, except in fish fed 10% body weight held at 27 °C (40% weight and 12% length increase). From preliminary chemical analyses, MeHg bioaccumulation was similar across feeding conditions but increased with temperature (~17-fold increase in MeHg concentration at 27 °C compared to a ~7-fold increase at 15 °C, regardless of feeding rate). The use of these data in a DEB model may aid in understanding why temperature is the dominant driver of MeHg accumulation at this scale, regardless of feeding or growth rate. Overall, this work contributes to the ongoing development of an ecological modeling framework in a fish species with an extensive toxicological and genomic background.

2938 PCB and Dioxin Exposure Increase Expression of CytochromeP4501A and the Aryl Hydrocarbon Receptor (AHR)-2 but Not AHR1 in Atlantic and Shortnose Sturgeons

M. DellaTorre[2], I. Wirgin[2], C. Chambers[1] and N. Roy[2]. [1]*National Oceanic and Atmospheric Administration, Highlands, NJ and* [2]*New York University, New York, NY.* Sponsor: T. Gordon.

Sturgeon populations are endangered worldwide and exposure to PCBs and dioxins is thought to be a major contributor to their decline. This issue is especially relevant in New York metropolitan waters because of the estimated 1.3 million pounds of PCBs that were released into the Hudson River between 1947 and 1977 and near world record levels of dioxin into the western estuary. However it is unknown which PCB congeners and what concentrations can potentially cause toxic effects in fishes. The goal of this study was to assess the risk of these contaminants for federally endangered Atlantic sturgeon *Acipenser oxyrinchus* and shortnose sturgeon *A. brevirostrum*, by determining the expression levels of cytochrome P4501A and the aryl hydrocarbon receptors (AHR) 1 and 2 which mediate toxicity of coplanar PCBs and dioxins through the AHR pathway. Fish were exposed as embryos for 24 hours to graded doses of coplanar PCBs 77, 81, 104, 126, 169 (from 0.01 to 10,000ppb), an Aroclor mixture (0.02 to 20,000ppb), or the most potent dioxin congener, TCDD (0.0001 to 100ppb). Once hatched, RNA was extracted from each individual larva and RT-PCR quantified the expression of the genes of interest. CYP1A and AHR2 increased with increasing concentrations for all contaminants except for PCB 104. This was expected because PCB 104 is a non-coplanar PCB meaning its structure makes it less likely to be an agonist to AHR and therefore less toxic. CYP1A induction was signif-

icant compared to controls by the 100ppb level for PCBs and at 0.1ppb for TCDD. AHR1 was not significantly increased in any of the contaminants at any concentration level, suggesting that AHR1 is less functional in mediating responses to these contaminants than AHR2. This research shows that sturgeons are sensitive to coplanar PCBs and TCDD at environmentally relevant concentrations. In addition, this study, by calculating species-specific Toxic Equivalency Factors (TEFs) for these PCB congeners, will help to provide accurate risk assessments of the impact of these PCBs and TCDD on Hudson River sturgeons and other sturgeon species worldwide.

PS 2939 Organochlorine Contamination of Fish in Lake Apopka

N. D. Denslow[1], V. Dang[1], K. J. Kroll[1], S. Supowit[2] and R. U. Halden[2]. [1]*Physiological Sciences, University of Florida, Gainesville, FL and* [2]*The Biodesign Institute, Center for Environmental Security, Security and Defense Systems Initiative, Arizona State University, Tempe, AZ.*

The North shore muck farms around Lake Apopka were used for heavy agriculture from the 1940's to 1990's. High levels of organochlorine pesticides (OCPs) were used for decades, contaminating the soil and the water in the area. Farmworkers were also sprayed while working on the farms and have complained of various ailments since that period, including lupus. To get a handle on the current levels of pesticides in Lake Apopka fish, we collected largemouth bass from various lakes in the region for contaminant analysis with a panel of pesticides. Fish from pristine reference lakes were below detection for the tested contaminants, but Lake Apopka largemouth bass continue to exhibit measurable levels of OCPs, 25 years after cessation of pesticide usage. Pesticides with measurable levels included aldrin, hexachlorocyclohexane (HCHs), p,p'-DDD, and p,p'-DDE. The tissue with the highest burden of total OCPs was the brain, followed by the liver and the gastrointestinal tract (0.23, 0.15 and 0.1 ug OCP/g wet weight fish, respectively). Lake Apopka largemouth bass were compared to experimental fish given a single oral dose of a mixture of p,p'-DDE and dieldrin at 10 ug/g fish for each. Fish given the oral dose were maintained in clean water conditions for 30 days after dosage. The experimental fish had highest burdens of contaminants in the gastrointestinal tract, followed by the bile and the liver (47, 27 and 14 ug/g wet weight fish, respectively). Trophic transfer studies suggest that OCPs are easily transferred from contaminated worms to fish through the diet. OCPs obtained through oral consumption are bioavailable and cause changes in the transcriptome in the liver of contaminated fish, possibly affecting reproduction. Fish consumption advisories have been lifted in Lake Apopka, but fish remain contaminated with OCPs.

PS 2940 Acute Toxicity of Graphene to Water Flea, Brine Shrimp and Zebrafish

S. A. Johari[2], S. Asghari[2], H. J. Ahn[1], E. K. Sohn[1] and I. J. Yu[1]. [1]*Hoseo University, Asan, Korea, Republic of and* [2]*1-Fisheries Department, University of Kurdistan, Sanandaj, Iran, Islamic Republic of.*
Sponsor: I. J. Yu.

In order to understand the potential ecotoxicity of Graphene released into aquatic environment, the toxicities of two types of this material were assessed using two freshwater (*Daphnia magna* and *Danio rerio*) and one saltwater (*Artemia franciscana*) organism. Two types of graphene were tested, including GPX-205 (750 m2/g, <2 micron lateral dimension) and GPX-103 (120-150 m2/g, 25 micron lateral dimension) provided by Cabot corporation. Based on the observations, water suspension of GPX-205 was very stable, while GPX-103 was not stable in water even after ultrasonication. According to the results, none of the tested substances caused the death of Zebrafish during 96 hours. Although GPX-103 didn't immobilize Daphnids during 48 hours, 48h-EC50 of GPX-205 was calculated as 17.519 mg/L for Daphnids. Also both materials caused signs of abnormal swimming behavior in *Daphnia*, especially swimming at the bottom. The 96h-LC50 of GPX-103 and GPX-205 for *Artemia* was calculated as 7.399 and 1.653 mg/L, respectively. Overall, acute toxicity of GPX-205 was higher than GPX-103; also sensitivity of brine shrimp was higher than water flea. More studies are needed to understand effects of long-term exposure of aquatic organisms to Graphene.

PS 2941 The Use of GPER and ER Antagonist to Evaluate the Mechanism of Bifenthrin Exposure in Embryos and Juveniles of Zebrafish (*Danio rerio*)

L. B. Bertotto[2] and D. Schlenk[2]. [1]*Environmental Sciences, University of California, Riverside, Riverside, CA and* [2]*Environmental Toxicology, University of California, Riverside, Riverside, CA.*

Bifenthrin (BF) is a pyrethroid pesticide widely used in urban and agricultural applications, having low acute toxicity to mammalian species and relatively low persistence in the environment. However, environmentally relevant concentrations of this compound enhanced plasma concentration of 17B-estradiol (E2) and altered the expression of dopaminergic pathway components in the central nervous system in fish. Significant decreases of GnRH2 and dopamine receptor 2A (DR2A) transcripts were observed when zebrafish (*Danio rerio*) embryos (3 hour post fertilization) were exposed to 1.5 ppb BF for 96 hours, and a significant increase in the expression of the tyrosine hydroxylase (TH) and dopamine active transporter (DAT) was observed when juveniles (one month old) were exposed to the 0.15 and 1.5ppb for 96 hours. Since dopamine regulates GnRH release, and BF has been shown to have antiestrogenic activity *in vitro*, embryo and juvenile (one month old) zebrafish were exposed for 96 hours and 2 weeks to a mixture of 0.15 and 1.5 ppb BF and an estrogen receptor (ER) antagonist (raloxifen). Likewise, a G-protein coupled estrogen receptor (GPER) antagonist (G15) with equal molar concentrations was used. The relative levels of transcripts related to the dopaminergic and the hypothalamic-pituitary-gonad axis (TH, DR2A, DAT, GnRH2, GnRH3 and vitellogenin) were investigated by qRT-PCR. Preliminary results show that the GPER antagonist in embryos had a greater impact than the ER antagonist. Further analysis of differentially expressed genes and signal transduction pathways will help assess potential toxic and sublethal effects of BF using zebrafish as an animal model.

PS 2942 3,4-Dichloroaniline, a Growth Inhibitor of Juvenile Zebrafish (*Danio rerio*)

J. R. Rana[2], D. Patel[2], M. Pandya[2], N. Khan[2], C. Callol[2] and V. J. Piccirillo[1]. [1]*VJP Consulting, Inc., Ashbum, VA and* [2]*Toxicology, Jai Research Foundation, Valvada, India.* Sponsor: V. Piccirillo.

Exposure of chemicals to the organisms may interfere with organismal integrity at the biochemical level and ultimately gives rise to effects at the individual level. These effects may result in reductions in ecologically relevant characteristics such as growth, reproduction and survival. Zebrafish has been a well-known vertebrate model in a variety of biological disciplines to assess drug toxicity and safety. Substantial information gathered from developmental and genetic research has placed zebrafish in an attractive position for use as a toxicological model. In this study, the effect of 3,4-Dichloroaniline (DCA), a metabolite of some herbicides and insecticide (propanil, diuron and diazinon), on the growth rate of juvenile zebrafish were assessed. Sixty days old juvenile zebrafish were exposed to various concentrations of DCA ranging from 0.016 to 0.5 mg/L along with control for 28 days. Study was performed under semi-static condition where media was renewed at every 24 hr intervals. The specific growth rate of fish exposed to the DCA test concentrations of 0.031, 0.063, 0.125, 0.250 and 0.500 mg DCA/L between days 0 and14; and 0 and 28 reduced significantly as compared with control group. All juvenile fish were normal and, no mortality was observed at the concentrations of 0.016, 0.031, 0.063, 0.125 and 0.250 mg DCA/L and in control group. The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) up to 28 day DCA exposure period were 0.016 and 0.031 mg/L, respectively. The EC50 values of DCA for average specific growth rate between days 0 and 14; 14 and 28; and 0 and 28 were 0.144, 0.178 and 0.257 mg/L, respectively. Based on the data, it can be concluded that DCA has no toxic effect on the growth of juvenile zebrafish at the test concentrations of 0.016, 0.031 and 0.063 mg DCA/L while DCA has toxic effect on the growth of juvenile zebrafish at the test concentrations of 0.125, 0.250 and 0.500 mg DCA/L up to day 28.

2943 Effects of Acute Exposure to Natural Estrogen on the Aggressive and Courtship Behaviors in Adult Male Zebrafish (*Danio rerio*)

G. Mercado[1] and L. Glazer[2]. [1]*Biology, Grinnell College, Grinnell, IA and* [2]*Biology, Woods Hole Oceanographic Institution, Woods Hole, MA.*

The focus of traditional sewage systems is the disinfection of wastewater before returning it back to the natural environment, while certain chemicals are not eliminated. Wild fish populations are vulnerable to many contaminants that are discharged from sewage systems and find their way into aquatic habitats. Endocrine disruptors are environmental pollutants that adversely affect hormonal systems within the body. One such chemical is the synthetic estrogen 17α-ethinylestradiol (EE2), an active component in oral contraceptives, which has been shown to exert harmful effects on the health, embryonic development and reproductive success of a number of fish species. In adult male zebrafish, acute exposure to EE2 resulted in reduced aggressive and courtship behaviors, indicating impairment in competitive and reproductive success. A global increase in the consumption of soy-containing supplements is occurring due to the advertisement of their potential health benefits. Soy contains phytoestrogens, which are a diverse group of naturally occurring compounds that are structurally similar to estradiol, and may therefore potentially act as endocrine disruptors with similar effects to those observed with EE2. Despite intensive research on the harmful effects of synthetic estrogens, very little is known on the impact of phytoestrogens. In order to examine the potential effects of soy-derived phytoestrogens on fish aggressive and courtship behaviors, we performed an acute, 5 hour, exposure of adult male zebrafish to ethanol (vehicle control), 50 pg/mL EE2 (positive control), and two concentrations of commercially purchased soy-extract; 0.8pg/mL (low concentration) and 4pg/mL (high concentration). We then compared the aggressive and courtship behaviors of the vehicle control and all three estrogen treatments of exposed fish. Our study suggests that phytoestrogens present in soy extracts that are sold commercially as dietary supplements may cause a reduction in male zebrafish aggression and courtship behavior. These results indicate possible negative impacts on the competitive and reproductive success of male zebrafish, which may be similar in wild fish populations that are exposed to estrogen discharges from sewage systems. [Support: Woods Hole Partnership Education Program]

2944 Assessment of Toxicity of Glufosinate Ammonium Using Early Stages of Zebrafish

F. Wang[1], X. Chen[1], D. Yang[1], J. Yang[1], G. Kang[1], B. Zhao[2], N. Zheng[4], M. Chernick[3], D. E. Hinton[3] and W. Dong[1,3]. [1]*Inner Mongolia Provincial Key Laboratory for Toxicants and Animal Disease/Inner Mongolia University for the Nationalities, Tongliao, China;* [2]*Institute of Pharmacology and Toxicology, Academy of Military Medical Science, Beijing, China;* [3]*Nicholas School of the Environment, Duke University, Durham, NC and* [4]*Northeast Institute of Geography and Agricultural Ecology, Chinese Academy of Sciences, Jilin, China.* Sponsor: W. Dong.

Glufosinate ammonium is broadspectrum non-selective herbicide. The biological safety attracts people's attention through increasing application. In this study, zebrafish embryo was used as an animal model and exposed with different doses of glufosinate ammonium and the mortality and morphological changes were monitored. In addition, we used techniques to investigate the effect of Vasa gene expression by *in situ* hybridization. As results, glufosinate ammonium caused death and induced curved tail and inhibited pigmentation. Furthermore, glufosinate ammonium also inhibited Vasa gene normal expression. These results indicated that glufosinate ammonium have lethal toxicity, teratogenicity and potential reproductive toxicity. This study shows the feasibility of using zebrafish embryo to evaluate the toxicity of pesticide.

2945 Functional Characterization of Glutathione-S-transferases Rho (Gstr1) and Theta 2 (Gstt2) in Zebrafish (*Danio rerio*) and Their Sensitivity to Environmental Toxicants

B. Glisic[1], J. Hrubik[1], E. Petri[1], A. Tubic[1], R. Zaja[3], T. Smital[3] and R. Kovacevic[1]. [1]*Department for Chemistry, Biochemistry and Environmental Protection, University of Novi Sad Faculty of Sciences, Novi Sad, Serbia;* [2]*Department of Biology and Ecology, University of Novi Sad Faculty of Sciences, Novi Sad, Serbia and* [3]*Laboratory for Molecular Ecotoxicology, Division for Marine and Environmental Research, Rudjer Boskovic Institute, Zagreb, Croatia.*

Glutathione-S-transferases (GSTs) represent a crucial part of cellular detoxification systems. The main function of GSTs is to alleviate the toxicity of diverse endogenous and exogenous compounds (xenobiotics). Rho and Theta class belong to the cytosolic family of GSTs in zebrafish (*Danio rerio*), an important vertebrate model species frequently used in environmental research [1]. The goal of our study was functional characterization of individual Rho (Gstr1) and Theta (Gstt2) enzymes including analysis of their interactions with specific environmental contaminants. Enzymes characterization was performed using recombinant expression in *E. coli* and subsequent protein purification. Enzyme kinetics were measured using GSH as co-substrate, and monochlorobimane and 1-chloro-2,4-dinitro benzene as model substrates. Evaluation of a series of potential inhibitors of GST enzymes that could be found in the environment, revealed enzyme-specific molecular interactions with tested compounds and enabled us to compare kinetic parameters with human and fish orthologs. Samples of untreated municipal wastewater downstream of urban discharge were collected and fractionated to obtain total extracts, non-polar, medium-polar, and polar fractions. Only the polar fraction inhibits activity of both enzymes (but in a different manner), while other fractions had no effect. In conclusion, characterization of Gstr1 and Gstt2 enzymes increases our understanding of their toxicological and physiological role(s) in fish and suggests their role as biomarkers. [1] Glisic B.; Mihaljevic I.; et al. (2015) Aquatic Toxicology, 158:50-62. Financial support: SNSF (SCOPES IZ73Z0_128025) and MESTD of the Republic of Serbia (173037).

2946 Effects of an Aqueous Coal Dust Extract on Developmental Toxicity and Gene Expression Profiling of Zebrafish (*Danio rerio*)

K. Caballero-Gallardo[2], S. E. Wirbisky[1], J. Olivero-Verbel[2] and J. L. Freeman[1]. [1]*School of Health Sciences, Purdue University, West Lafayette, IN and* [2]*School of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia.*

Coal is a major energy source that generates diverse environmental impacts through all its productive chain, mostly by the release of coal dust particles. An aqueous coal dust extract was obtained from a mineral sample taken from one of the largest coal mines in Colombia. Trace elements were quantified in the extract by ICP/MS, and its toxicity evaluated using zebrafish (*Danio rerio*). Fish embryos were exposed to different concentrations of aqueous coal extract (0, 0.1, 1, 10, 100 and 1000 µg/mL) to measure lethality as well as morphological and transcriptomic alterations. The extract showed Sr, Zn, Ba, As, Cu, Se, Li, Ni, Sb, Rb, Co, and Cr had the highest concentrations. No significant difference was observed in mortality during the experiment with survival near 90% in all treatments ($p>0.05$). A significant decrease in the percent of embryos hatched was observed in the 0.1 and 1000 ppm treatment groups at 72 hours post fertilization ($p<0.0001$). No significant differences were observed in any of the developmental measurements in any of the treatments ($p>0.05$). Results from the microarrays revealed changes for 77, 61, and 1376 genes in the 1, 10, and 100 ppm treatments, respectively. Altered genes were associated with physiological system development and function. In all tested extract treatments altered genes were related to biological processes such as apoptosis, cell cycle, and cellular processes, among others. In short, these findings are the first to identify genetic and molecular targets altered in response to the exposure to an aqueous extract of coal. Colciencias (Colombia): 1107-569-33952 and 567-2012.

2947 Effects of Tris(2-Butoxyethyl) Phosphate (TBEP) on Endocrine Systems and Reproduction in Zebrafish (*Danio rerio*)

H. Shin[1], K. Kim[1] and K. Ji[2]. [1]*Seoul National University of Science and Technology, Seoul, Korea, Republic of and* [2]*Yongin University, Yongin, Korea, Republic of.* Sponsor: K. Kim.

Tris(2-butoxyethyl) phosphate (TBEP), one of widely used organophosphate flame retardants (OPFRs), has been frequently detected both in environment and biota. However, potential effects of TBEP on endocrine system and its underlying mechanism have not been examined. We exposed TBEP to adult zebrafish pairs at 0, 2.1, 11, 118 µg/L for 21 d, and investigated the effects on gene transcriptions and hormone production belonging to the hypothalamic-pituitary-gonad (HPG) axis and reproduction. The adverse effects on F1 generation were further examined. We found that TBEP exposure led to significant decrease in average egg production. In male fish, plasma concentrations of 17β-estradiol were significantly increased along with up-regulation of cyp19a. Expression of key transcription factors regulating steroidogenesis was affected by TBEP exposure in brain and testis in male fish, and ovary in female fish, respectively. Parental TBEP exposure to F0 generation resulted in delayed and lesser rates of hatching in F1 generation. This study demonstrates that the exposure of low level of TBEP could affect the sex hormone balance by altering regulatory circuits of the HPG axis, eventually leading to disruption of reproductive performance and the development of offspring. The potential in adverse effect of TBEP in the environment, being masked under increasing production as brominated flame retardant replacement, therefore, should be taken into consideration. This study was supported by National Research Foundation of Korea (NRF, Project no. 2015R1C1A1A02037063)

2948 Analysis of Toxic Effects Using Differential Gene Expression in the *Danio rerio* Embryo Test

W.-K. Kim[1] and J. S. Choi[2]. [1]*Korea Institute of Toxicology, Daejeon, Korea, Republic of and* [2]*Korea Institute of Toxicology, Jinju, Korea, Republic of.*

The zebrafish (*Danio rerio*) embryo toxicity test (DarT) is under consideration as a replacement method for the acute fish toxicity test. However rarely reveal mechanisms leading to toxic effect and are relatively insensitive compared to chronic toxic effects. Therefore, gene expression could be used to predict chronic fish toxicity, the zebrafish (*Danio rerio*) embryo test (DarT), was expanded to a gene expression *D. rerio* embryo test (Gene-DarT). The Early life stage test (ELST) was performed according to the OECD guideline210 (OECD210, 2013). Embryos were exposed to 0.0625, 0.25, 1, 4 and 16 mg/L ZnSO4, we identified a LOEC of 1mg/L. The effects of ZnSO4 on lethal and sublethal endpoints of the DarT and on expression of potential marker genes were investigated by oligonucleotide array: Three genes involved in taar10c, camkv1 and irf2a were significantly regulated. After exposure of zebrafish embryos for 48h, differential gene expression was evaluated using qPCR. Lowest-observed-effect concentrations (LOECs) values derived with the Gene-DarT differ by a factor of less than 15 from LOEC values of fish early life stage test with zebrafish. And the Gene-DarT was by a factor of more than 100 less sensitive than the DarT. The results of this study indicate that analysis of gene expression could be used to predict effect concentrations in the fish early life stage test.

2949 Effects of 2- and 6-Hydroxylated Chrysene on the Development of *Danio rerio* Embryos

G. Diamante[2], G. A. Müller[1], A. C. Bainy[1] and D. Schlenk[2]. [1]*Department of Biochemistry, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil and* [2]*Environmental Toxicology, University of California, Riverside, Riverside, CA.*

Oil spills are one of the primary sources of polycyclic aromatic hydrocarbons (PAHs) in marine environments. They are subject to biotic and abiotic weathering that can alter their physical and chemical characteristics. PAHs can undergo photochemical oxidation forming oxygenated photoproducts that can have severe effects on marine life and the environment. Among the PAHs found in crude oil, chrysene is one of the most persistent in the water column and can undergo photo-oxidation to produce oxygenated derivatives such as 2-hydroxychrysene and 6-hydroxychrysene. Both of which have been shown to have estrogenic effects that can potentially disrupt estrogen-signaling pathways important during development. To evaluate this, *Danio rerio* embryos (2 hpf) were exposed to 2- and 6-hydroxychrysene separately for 74 hours under controlled laboratory conditions. Embryos were treated with several concentrations of 2-hydroxychrysene (0.1 µM, 0.5 µM, 1 µM, 2 µM, 5 µM) and 6 -hydroxychrysene (0.5 µM, 3 µM, 5 µM, 8 µM) to determine percent survival and the occurrence of deformities. Our data show no significant difference in mortality after exposure to 2-hydroxychrysene at 76hpf; however, there was a significant decrease in the survival of the larvae, 4 days after treatment. There was also a significant increase in the percentage of cardiac deformities observed, 34 ±20.1%, 62 ±29.2%, 92 ±14.1% and 97 ± 5.44%, after exposure to 0.5, 1, 2, and 5µM of 2-hydroxychrysene, respectively. When embryos were treated with 3 µM of 6-hydroxychrysene the percent survival was 65 ±3.9% at 76hpf. The occurrence of cardiac deformities was also significantly higher after treatment with 6-hydroxychrysene compared to the control group. After exposure to 3 µM, 5 µM and 8 µM of 6-hydroxychrysene, the percentages of cardiac deformities were 61 ±19.4%, 68 ±17.4% and 67 ±17.1% respectively. These studies show for the first time the regioselective impacts of hydroxylated PAHs on cardiac development and survival of fish embryos. Our findings raise the need to identify mechanisms that are involved in the toxicity of these compounds to assess the potential risks of oil spills on fish populations. This research was made possible in part by a grant from BP/The Gulf of Mexico Research Initiative to the RECOVER Consortium, and in part by CAPES/INCT-TA and CNPq.

2950 Copper Pyrithione Induces Abnormal Muscle and Notochord Architecture in Developing Zebrafish Embryos

K. Almond and L. Trombetta. *St. John's University, Queens, NY.*

A major concern for alternatives to organotins in marine biocides, particularly in paint, is the lack of data on non-target marine organisms. Since the strict regulatory use of antifouling agents has been imposed, companies have been required to find effective and less toxic alternatives. A frequent substitute within the Asian market has been the metal pyrithiones, principally zinc and copper. Copper pyrithione (CuPT), an active component in antifouling biocides, has been shown to cause anomalies during early development of the mummichog, *Fundulus heteroclitus*. Zebrafish, *Danio rerio*, embryos were exposed within the first hour after fertilization to 12 and 64 µg/L of CuPT for 24 hours. Morphological abnormalities of the notochord and muscle architecture were observed at 96 hours post fertilization (hpf). Animals exposed to 64 µg/L of CuPT resulted in a severe twisting of the notochord and a curved body axis. Transmission electron microscopy revealed abnormal electron dense deposits in the notochord sheath and muscle fiber degeneration in animals treated with 12 µg/L of CuPT. Embryos that were exposed to 64 µg/L of CuPT showed more severe muscle fiber degeneration including abnormal A and I band patterning and altered z disc arrangement. Abnormalities in the notochord sheath, swelling of the mitochondria and numerous lipid whorls were also seen. Acridine orange staining revealed an increase in apoptosis particularly in the brain, eye, heart and tail regions of both treated groups. Apoptosis was confirmed with an increase in caspase 3/7 activity in both treatment groups. These results demonstrate that copper pyrithione causes severe alterations in morphogenesis. Exposed zebrafish embryos showed ultrastructural hallmarks of peroxidative injury and cell death via apoptosis. These changes question the use of copper pyrithione as an antifouling agent.

2951 Characterization of Cardiac Deformity, CYP Induction, and Developmental Bioenergetics Following PAH Exposures in PAH-Resistant and Non-Resistant *Fundulus heteroclitus*

C. D. Lindberg[2], N. Jayasundara[2], J. S. Kozal[2] and R. T. Di Giulio[2]. [1]*Integrated Toxicology and Environmental Health Program, Duke University, Durham, NC and* [2]*Nicholas School of the Environment, Duke University, Durham, NC.*

Sites along the Elizabeth River (Virginia, USA) are contaminated with polycyclic aromatic hydrocarbons (PAHs) due to anthropogenic activities. Populations of Atlantic killifish (*Fundulus heteroclitus*) inhabiting these areas are resistant to PAH-induced teratogenesis. In this study, we compared subpopulations of killifish that inhabit a creosote contaminated site, Republic (Rep), and pristine site, Kings Creek (KC). We quantified developmental cardiotoxicity, induction of cytochrome P450 1A (CYP1A), and bioenergetics in KC and Rep embryos following exposure to PAH mixtures. At 24hpf, embryos were treated with Elizabeth River sediment extract (ERSE) (1%, 3%, 5% or 10%), mixtures of benzo-a-pyrene (BaP) and fluoranthene (FL) (50+250µg/L or 100+500µg/L BaP+FL) or controls (0.1% DMSO or artificial seawater). In ovo CYP1A activity was measured with ethoxyresorufin-o-deethylase (EROD) at 4dpf; at 6dpf cardiac deformities were scored. Using the XFe24 Extracellular Flux Analyzer (Seahorse), we examined differences in basal respiration rates (oxygen consumption rate (OCR)) between control embryos and those exposed to 1% ERSE. KC embryos had significantly elevated CYP1A activity after all exposures. Relative to KC, Rep embryos were

recalcitrant to CYP1A induction following BaP+FL exposures, but not ERSE exposures. PAH exposures induced cardiac deformities in KC, but not Rep embryos. Together, these results suggest that cardiotoxicity of ERSE is possibly mediated via a CYP-independent mechanism in killifish. Basal OCRs of Rep embryos were elevated relative to KC after Rep OCRs diverged from KC at 32hpf. Treatment with 1% ERSE also elevated OCRs for both subpopulations at most time points. This is the first report of F. heteroclitus embryonic basal bioenergetic profiling. Due to their relatively extended embryonic development (14 days), killifish may be a useful model to explore mitochondrial biogenesis, function, and toxicant-induced dysfunction. This work was supported by NIEHS T32-ES021432 and P42ES010356.

2952 A Multi-Organ Approach for Studying the Impacts of the Polycyclic Aromatic Hydrocarbon Phenanthrene on the Reproductive Axis

J. R. Loughery[1], E. Crowley[1], A. Mercer[1], K. A. Kidd[1] and C. Martyniuk[2]. [1]*Department of Biology, University of New Brunswick, Saint John, NB, Canada and* [2]*Department of Physiological Sciences, University of Florida, Gainesville, FL.* Sponsor: N. Denslow.

Phenanthrene (PHEN) is a polycyclic aromatic hydrocarbon (PAH) found in the environment due to human activities such as oil extraction and coal mining. PAHs induce oxidative stress, and impair immune and reproductive systems in fish and mammals. The objective of this study was to determine the effects of PHEN on the reproductive axis of the toxicological fish model (FHM, *Pimephales promelas*). Female FHM were exposed to an average measured concentration of 201.8 µg/L PHEN or a control for approximately 2 months. Ovaries were excised and assessed for *in vitro* 17β-estradiol (E2) production and histological analysis. The liver and hypothalamus were collected for transcriptomic analyses. PHEN treatment decreased gonad size compared to control fish. PHEN also decreased the proportion of vitellogenic oocytes, demonstrating that PHEN disrupted the development of female gametes. E2 production was not affected by PHEN exposure. In the liver, gene expression analysis determined that there were 628 differentially expressed probes (DEPs) affected by PHEN. The genes included MHC class I antigen, and epidermal growth factor receptor. Sub-network Enrichment Analysis of human homolog genes suggested that there was an increase in transcripts related to energy utilization while pathways related to immune system function were decreased by PHEN. Transcriptomic profiling in the hypothalamus determined that there were 1150 DEPs, and only 65 were common to the hepatic response. Sub-network Enrichment Analysis determined that estrogen receptor signaling pathway, triacylglycerol metabolism, and dopamine metabolism pathways were regulated by PHEN. This study corroborates others that show sub-chronic exposures to PHEN can impair reproductive processes. It is hypothesized that FHMs are utilizing energy resources to mitigate stress responses due to PHEN exposure, subsequently reducing energy available for reproduction.

2953 Benzo[a]pyrene Modulates Changes on Stages of Development and in Genes Related with DNA Methylation in Male Tilapia, *Oreochromis niloticus*

N. A. Albornoz-Abud, O. Zapata-Pérez and R. C. Colli-Dula. *Marine Resources, Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Unidad Mérida, Mérida, Mexico.*

Polycyclic aromatic hydrocarbons (PAHs) have been shown to cause significant effects in fish, such as changes in xenobiotic metabolism as well as physiologic processes required for survival, development and reproduction with most of these changes being caused by altered gene expression. Recently, it has been reported that Benzo[a]pyrene (BaP) can alter expression of several DNA methylation enzymes including Ten eleven translocation enzymes (TET) most specifically methylcytosine dioxygenases (TET1 and TET3) in fish. TET proteins are involved in important biological processes, including DNA demethylation and transcription, embryonic development, stem cell function, and cancer generation among others. However, their involvement with gene regulations in different stages of sexual development is unknown. In this study we used morphometric responses to evaluate general health and stages of sexual development as well as qPCR analysis to determine changes in the expression of key genes related with DNA methylation enzymes in Nile tilapia, following of a single sublethal dose of BaP. Both male juvenile and adults tilapias were injected intraperitoneally with 40 mg/kg of BaP and kept under laboratory conditions for 60 d. Fish were sacrificed and target tissues (liver, gonad and kidney) were collected at day 1, 2, 3, 5 and 60 for posterior analysis. Hepatosomatic, gonadosomatic and the nephrosomatic indices were not altered significantly at any time point. Condition factor was significantly decreased in BaP-treated fish after 5 and 60 d compared to controls. Changes in the gene expression of DNA methylation enzymes could be related with alteration on their development. These results provide insight into BaP mechanisms associated with epigenetic modifications by identifying potential altered genes and their links with physiological responses in both juvenile and adult stage Nile tilapia.

2954 Diazinon-Induced Clastogenity and Pathological Changes in Ovaries and Testes of *Clarias gariepinus*

O. E. Ola Davies[2], A. F. Fagbohun[2], B. O. Emikpe[2] and O. K. Adeyemo[2]. [1]*Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria and* [2]*Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria;* [3]*Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria.*

In most developing countries, one of the problems facing aquaculture industry is the pollution of ponds and rivers with pesticides. Chemicals such as diazinon, an organophosphate pesticide, originating from agricultural activity enter the aquatic environment through atmospheric deposition, surface run-off or leaching. In a triplicate experimental set-up, seventy - two (72) apparently healthy catfish comprising adult and juvenile of both sexes were exposed to a previously determined "no effect concentration" (0.405ppm) of diazinon. Another set of fish was exposed to 0.0625µg Sodium arsenite, a known clastogen, which was used as the positive control, while another set of catfish exposed to the culture water alone were the negative control. Between 24 and 48 hours of exposure, micronuclei induction in polychromatic erythrocytes was determined in subsets of experimental groups, while exposure continued for 28 days. Catfish's sex organs were harvested on days 21 and 28 to determine the effect of long-term exposure to diazinon on histology. Water quality was also monitored. The result established a significantly higher mean Micronucleated Polychromatic Erythrocytes (15) in catfish exposed to diazinon suggesting genetic damage (normal is ≤ 4). The MnPCE in Sodium arsenite exposed fish was 28, while that of the control group was below 4. Histological alteration observed in the ovary and testis was distorted matured cells and extensive testicular degeneration, respectively. The results showed that diazinon has clastogenic effect, and may have endocrine disrupting properties because of the histological changes observed in the ovaries and testis.

2955 An Integrated Study on DDT and its Metabolites in Sediments of Palos Verdes, California (USA): *In Vitro* Bioassessment of Trophic Transfer and Comparisons with Field Monitoring of Residues and Effects in Male Hornyhead Turbot (*Pleuronichthys verticalis*)

G. Xu[2], J. Crago[2], A. Kupsco[2], F. Jia[2], A. Mehinto[1], W. Lao[1], K. Maruya[1], J. Gan[2] and D. Schlenk[2]. [1]*Southern California Coastal Research Project, Costa Mesa, CA and* [2]*University of California, Riverside, Riverside, CA.* Sponsor: D. Schlenk.

High concentrations of DDT and its metabolites have been detected in sediment and hornyhead turbot (*Pleuronichtys verticalis*) collected from the Palos Verdes (PV), California, USA. The goal of this study was to assess transfer of DDT metabolites and their biological activities from sediment to polychaete (*Neanthes arenaceodentata*) to turbots, and to investigate if the responses in turbots from two laboratory exposures (i.e., 7 and 28 days) mimic those of field caught turbots. After 7 days exposure, male hornyhead turbot fed 3g-. and 10g of worm weight/kg of turbot body weight of PV sediment exposed worms showed an increase in total DDT metabolite concentration in the liver as compared to the control, and treated animals were equivalent to liver values from hornyhead turbot caught in the PV. DDT metabolites also accumulated in livers of male hornyhead turbot gavaged every other day for 28 days with a DDEs, DDDs, and DDT equaling an equivalent of being fed 30g of worm wt. /kg of turbot body wt. of PV sediment exposed worms. *In vitro* cell indicated significant increases of aryl hydrocarbon toxicity equivalents (TEQ, avg 13.21 ± 16.93 pg/g) and 17β-estradiol equivalents (EEQ, avg. 4.71 ± 1.12 ng/g) in bile extracts of turbots fed 10g/kg worm as compared to the control in the 7-day study. These values corresponded to those in the bile samples from fish caught from PV. Higher expression of hepatic CYP1A1, VTG, ERα and ERβ mRNA were found in livers of turbot caught from PV than those from the San Diego reference site. Liver extracts from the 28-day DDT-only treatment showed lower TEQ and EEQ than liver samples from fish caught from PV but both hepatic VTG and ERβ were significantly upregulated in both treatments. These data

indicated that trophic transfer of DDT-contaminated sediment for 7 days in laboratory conditions corresponded to field measurements of DDT residues and multiple bioassays suggested contributions of DDTs (at relatively high dose and long duration) to estrogenic effects observed in the PV superfund sites. (NIEHS Grant No: R01ES20921).

PS 2956 *In Silico* Interaction Analyses between α-Pinene and Orthologous Cyp6dg1v1 or Cyp6dg1v3 from Bark Beetles Dendroctonus rhizophagus and *D. valens* (*Curculionidae: scolytinae*)

M. F. Lopez-Gomez[2], G. Ramirez-Salinas[1], J. Correa-Basurto[1], G. Zuñiga[3] and A. Albores[2]. *[1]Laboratorio De Modelado Molecular, Escuela Superior De Medicina, IPN, Mexico City, Mexico; [2]Toxicology, Centro De Investigacion Y De Estudios Avanzados Del IPN, Mexico City, Mexico and [3]Zoologia, Escuela Nacional De Ciencias Biologicas, IPN, Mexico City, Mexico.*

The genus *Dendroctonus* includes the main pine-killing species in the world. Bark beetle life cycle occurs under the bark of pine trees, where feeds phloem and breed. During the host colonization, they should overcome the chemical defense system of trees, integrated by resin´s terpenes, diterpenes and sesquiterpenes. Some terpenes attract insects, but also are very toxic to them. Experimental evidence suggests that three insect gene families: cytochromes P450, esterases and glutathione-S-transferases, are involved in toxic xenobiotics metabolism. To understand the interaction between α-pinene and orthologous CYP6DG1, we report here an *in silico* study employing docking and molecular dynamic simulation (MD). The MD simulations of CYPs during 500 ns show that both proteins undergo structural conformation changes that reach a particular conformation with heme at 280, 340 and 370 ns time points, these changes are reflected at F86, A87, V279, A280, T284, P345, L346, L347 and L461 residues, all of them located at the substrate recognition site, and contribute to the formation an hydrophobic net that allows the enzyme interaction with α-pinene by means of electrostatic interactions. Our results show that CYP6DG1 enzymes might be involved in kairomones biotransformation. The information offered here may help to reach a better understanding of the insects CYPs interactions with monoterpenes involved in the bark beetle trees colonization, a relevant part of the insects' life cycle. (Supported by Conacyt: 175839 postdoctoral grant to MFLG and project Salud 162391; and also to H Oliver-Hdez, Hybrid Cluster Supercomputer "Xiuhcoatl", Cinvestav).

R 2957 Combination Toxicology: Are We Testing the Right Things?

L. A. Burns Naas[1] and H. Haggerty[2]. *[1]Gilead Sciences, Inc., Foster City, CA and [2]Bristol-Myers Squibb Company, New Brunswick, NJ.*

Whether it is to increase efficacy by targeting multiple pathways for the same disease or to improve safety by being able to lower doses of one or more drugs, the use of combinations of drugs in clinical development is increasing. Global regulatory guidance has provided a framework for the nonclinical safety evaluation of combination products, which considers the need for testing based on such things as the potential for PK or PD interactions, overlapping toxicology profiles, extent of toxicology characterization of the individual agents and their margins of safety, human experience with the individual agents, stage of development, etc. Notably, combination testing of drugs intended for advanced oncology indications is typically not warranted. Rather, sponsors are encouraged to obtain toxicology-related endpoints from pharmacology studies to inform the need for additional testing. This leaves open the possibility that unexpected adverse events associated with targeted, novel agents could be observed clinically. The objective of this deliberately provocative session is to provide a forum to begin to discuss the possibility that the current testing paradigms may be less useful than originally envisioned or may not be providing sufficient safety evaluation for the clinical assessment of combination drugs. The session will consider examples in which testing under existing guidance has had limited to no clinical impact, as well as examples in which potential safety issues were identified in situations where testing may not have been warranted under existing guidance. A proposal for a rational paradigm on how to address potential safety liabilities of particular drug combinations, and a framework for deciding when and how to interrogate *in vitro* or *in vivo* preclinical models in addressing combination safety will be presented by the third speaker. Examples presented will discuss incorporating safety criteria that will minimize combination toxicities for a particular target, explorating potential synergistic toxicities in the combination of two redundant targets, and developing a preclinical model for dose-limiting clinical combination toxicity and using this model to evaluate whether an alternative target would mitigate the toxicity. The final speaker will provide a regulatory perspective on the adequacy of the current testing for oncology indications including the rationale for existing regulatory guidance. The following questions will be posed during the roundtable to facilitate the initial discussion: Is the current testing paradigm providing the appropriate investigation and adding to our understanding of the potential safety concerns for combination products? What is the most appropriate dose of each agent to test in order to adequately characterize the combination safety and protect patients? Given the limitations often encountered with crossreactivity and different animal models of disease in oncology that may impact the ability to draw concrete conclusions from these efficacy models, should greater consideration be given to evaluating the nonclinical safety of combinations of oncology drugs? If yes, is an otherwise healthy animal the appropriate model?

IS 2958 Updating FDA's Redbook: The Importance of Stakeholder Involvement

D. Keefe[1] and Y. J. Zang[2]. *[1]US FDA, College Park, MD and [2]US FDA, College Park, MD.*

The US Food and Drug Administration (US FDA) is updating its guidance titled "Toxicological Principles for the Safety Assessment of Food Ingredients," known less formally as the "Redbook." US FDA proposes to expand the scope of the Redbook to include chemical safety assessments for all products over which US FDA's Center for Food Safety and Applied Nutrition has statutory authority, including regulatory contexts such as food additives, food contact substances, dietary supplement ingredients, food contaminants, and cosmetics. This information session will introduce the proposal and engage the audience in a discussion as to how to best facilitate stakeholder involvement. The introductory presentation will give an overview about the agency's considerations underlying this proposal. With the increase in public awareness of food safety issues, US FDA must ensure its toxicology toolbox keeps pace with advances in science and technology. The time is right for US FDA to ask the question of whether or not the guidance in the Redbook used in the assessment of food ingredient safety represents an articulation and application of the best science to garner an understanding of the toxicity profile of food ingredients. US FDA recognizes the importance of stakeholder involvement in updating the Redbook and will outline its strategies for ensuring transparency and the inclusiveness of all interested parties in this endeavor. The second presentation will discuss the process by which US FDA plans to update the Redbook. The updated project is complex and large in scope. US FDA must ensure that actionable guidance, developed with extensive input from stakeholders, begins to become available as soon as reasonably possible. At the same time, stakeholders must have adequate information to understand the overall direction of the project and the context within which individual sections of the guidance will function. US FDA's plans for a "roadmap" of the overall project in conjunction with prioritization of individual sections for publication will be discussed. The third presentation will emphasize that guidance on methodologies to assess food ingredient safety must include the most up-to-date scientific approaches that will most accurately assess the safety. New knowledge and toxicological approaches have the potential to make risk assessment more mechanistically based. However, whether these new approaches are ready to translate into changes in regulatory policies that govern human risk assessments needs to be thoroughly evaluated. Criteria for when a particular new toxicology method is validated sufficiently that it may be considered for incorporation into guidance will be discussed. The last presentation will share highlights of the International Life Sciences Institute (ILSI) North America's recommendations for Redbook enhancement from a stakeholder's perspective. Based on work undertaken by the ILSI organization, this presentation will discuss components of the Redbook that should receive the highest priority for review, new topics that are currently not addressed and should be considered for incorporation into the Redbook, and aspects of how the Redbook can be updated to more fully support the development and submission of safety assessments for substances introduced into food. As a stakeholder, ILSI believes this transparent and iterative process of the Redbook revision offers an opportunity for the US FDA to demonstrate global leadership and provide a model for global adoption of safety assessment for substances.

2959 Why Did the Scientific Program Committee Reject My Proposal? Developing a Good Idea into an Accepted SOT Session

J. B. Morris[1] and P. E. Ganey[2]. [1]*University of Connecticut, Storrs, CT and* [2]*Michigan State University, East Lansing, MI.*

The scientific sessions at the Annual Meeting are selected from proposals submitted by the membership. In this way the Scientific Program Committee (SPC) ensures the Annual Meeting Program is responsive to the overall membership needs. Every year, many more proposals for sessions are received than could possibly be scheduled in the Annual Meeting. Thus, less than one-half of the proposals submitted for scientific sessions (symposia, workshops, informational sessions, roundtable sessions, regional interest sessions, and historical highlights) are accepted. Although many of the rejected proposals focus on a highly pertinent topic, they ultimately fail because of weaknesses in critical aspects of the submission. The SPC, which carefully considers input from SOT Specialty Sections, Special Interest Groups, and Committees (i.e., "component groups"), is responsible for selection of the scientific sessions that are presented at the Annual Meeting. This informational session is being hosted by the SPC with the objective to help members develop high-quality proposals for these scientific sessions. Participants will learn about the process by which proposals are selected to gain an appreciation for the value of working with one or more component groups during the development stage. Aspects of proposals that are critical to acceptance (thorough development of abstracts, balance among speakers, etc.), as well as those that can reduce enthusiasm, will be discussed. The session will conclude with a 20-minute question and answer period during which session attendees will be encouraged to address the speakers, all of whom are members of the SPC, in an informal setting. This session supplements the "Best Practices—Proposal Submission and Review" webinar hosted by SOT for component groups at the beginning of March.

2960 The Evolution of the Postdoc: Transitioning from Trainee to Professional in the Modern Era

K. E. Sant[1] and S. Snow[2]. [1]*University of Massachusetts, Amherst, MA and* [2]*US EPA, Research Triangle Park, NC.*

The postdoctoral experience has changed considerably over the past decade. The number of postdocs in the United States has been consistently growing, as has the time spent in this transitional position. The average age at which scientists are appointed to their first faculty job and awarded their first NIH grant has been increasing. This "hypercompetitive" culture in biomedical research has resulted in several publications and reports by the National Academies and the National Postdoctoral Association that focused on the best practices needed to improve the quality of the postdoctoral experience. This session is designed to bring leaders from across various research sectors to discuss what it means to currently be a postdoc in an academic, government, and industry setting. Speakers will focus on (1) the modern state of postdocs in their sector; (2) the advantages/disadvantages of pursuing a postdoctoral fellowship in their sector; (3) the postdoc as a mentored position; and (4) how we move forward to produce a high-quality, sustainable workforce. Each speaker will discuss how their sector is addressing the current needs of postdocs and the workforce, and will provide the audience with their thoughts on how to navigate to different career paths. Speakers will also address how a postdoc in their sector may help produce a diversified workforce. This discussion will be highly relevant to all student and postdoctoral attendees, as well as senior scientists currently mentoring trainees. This career development session will inspire toxicologists to think critically about their training and to develop an improved roadmap to navigate to their ideal careers.

2961 Geographic Variations in Female Breast Cancer Incidence in Association with Emissions of Polycyclic Aromatic Hydrocarbons

C. Large[2] and Y. Wei[2]. [1]*Mercer University School of Medicine, Macon, GA and* [2]*Department of Community Medicine, Mercer University School of Medicine, Macon, GA.*

A geographical variation of breast cancer incidence exists, with incidence rates being much higher in industrialized regions. Though the risk for breast cancer is multifactorial, environmental factors such as exposure to ambient air pollution, specifically polycyclic aromatic hydrocarbons (PAHs), may play an important role in its etiology. In this study, we examined the difference in female breast cancer incidence between northeastern and southeastern regions of the U.S. and assessed the role that PAHs may be playing in the incidence of breast cancer by analyzing the data from the Surveillance, Epidemiology, and End Results Program (SEER) and Environmental Protection Agency. Linear regression analysis was conducted to evaluate the association between PAHs emissions and breast cancer incidence in unadjusted and adjusted models using SPSS 21. Significantly higher incidence rates of female breast cancer were seen in northeastern SEER regions, when compared to southeastern regions, as were higher levels of PAHs emission density also seen in the northeastern region. Linear regression analysis revealed that PAHs emission density was positively associated with annual incidence rates of breast cancer among the northeastern and southeastern states ($r = 0.584$, $\beta = 0.541$, $p = 0.009$). When adjusted for potential confounders, such as state poverty levels, race (non-Hispanic white), and state education levels, the associations remained to be statistically significant ($\beta = 0.551$, $p = 0.005$). This study suggests that the higher incidence of female breast cancer in the northeastern United States may be associated with the higher emissions of carcinogenic PAHs in this region. Further investigations are warranted to explore this relationship.

2962 Could the Adoption of a Chemical Release Prevention Program Have Prevented the Elk River Chemical Leak?

M. Nye, T. L. Knuckles and M. McCawley. *Occupational and Environmental Health Sciences, West Virginia University, Morgantown, WV.*

In January 2014, 4-methylcyclohexanemethanol (MCHM), a coal processing chemical with limited toxicological data, contaminated West Virginia's largest drinking water supply. The purpose of this study was to examine the effectiveness of the West Virginia Chemical Release Prevention Program (CRPP) in assessing risk when limited toxicological information exists. Mixture identity, release amount, and lack of comprehensive toxicological data presented emergency responders with great challenges in protecting public health. At the state government's request, the Centers for Disease Control (CDC) established a drinking water exposure threshold of 1 ppm for MCHM. However, the CDC neither considered inhalation nor potential subacute exposure. Furthermore, additional tests by the USGS found methyl-4-methylcyclohexanecarboxylate (MMCHC) in the drinking water, a mixture component for which no standards were adopted. The leak caused the state to resurrect an outstanding U.S. Chemical Safety Board recommendation that calls for enacting a program to prevent chemical releases and optimize emergency response. Characteristics for determining hazard considering chemical lifecycle and routes of exposure have been outlined in a proposed CRPP implementation plan. In this study, epidemiological data were synthesized and a literature review was performed on studies specific to the Elk River chemical leak in order to identify pertinent risk assessment gaps. This was compared to methods described in the CRPP plan. Findings indicate that the implementation plan identifies methodology that could have assisted government officials and emergency responders in more immediately characterizing risk of the Elk River chemical leak. The CRPP implementation plan offers a new risk assessment and methodology including broad grouping of an agent's effects, a risk matrix and an assessment of the likelihood of a potential chemical release when the toxicological profile of a chemical is limited.

2963 Birth Outcome Measures and Prenatal Exposure to 4-tert-octylphenol Among Chinese Babies

S. Lv[1,2], C. Wu[2], X. Qi[2,3], X. Chang[2], J. Guo[2] and Z. Zhou[2]. [1]*Sahnghai Center for Disease Control and Prevention, Shanghai, China;* [2]*School of Public Health, Fudan University, Shanghai, China and* [3]*Zhejiang Center for Disease Control and Prevention, Hangzhou, Zhejiang Province, China.*

4-tert-octylphenol (tOP) is known as intermediate material and main degradation product of octylphenol polyethoxylates, which are nonionic surfactants extensively used as components of plastics, detergents, paints and pesticides. Exposure to tOP has been linked with adverse health outcomes in animals and humans, while epidemiological studies about associations between prenatal exposure to tOP and fetal growth are extremely limited. We measured urinary tOP concentrations with large-volume-injection gas chromatography tandem mass spectrometry in 1100 pregnant women (average age, 26 ± 5.4 years) before their delivery, and examined whether their tOP levels were associated with birth outcomes, including weight, length, head circumference and ponderal index at birth. tOP could be detected in all samples, and the median uncorrected and creatinine-corrected tOP concentrations were 0.90 μg/L (range from 0.25 to 20.05 μg/L) and 1.33 μg/g creatinine (range from 0.15 to 42.49 μg/g creatinine), respectively. The proportions of male and female neonates were similar. Average birth weight was 3.49 ± 0.44kg, average birth length was 51.2 ± 2.3 cm, average head circumference was 34.7 ± 1.4 cm and average PI was 2.60 ± 0.32 g/cm3. Maternal urinary log-transformed tOP concentrations were significantly

negatively associated with adjusted birth weight [β (g) = -126; 95% confidence interval (CI): -197,-55], birth length [β (cm) = - 0.53; 95% CI:-0.93,-0.14], and head circumference [β (cm) = -0.30; 95% CI:-0.54, -0.07], respectively. Additionally, considering sex difference, these significant negative associations were also found among male neonates, while only higher maternal tOP concentrations were associated with a significant decrease in birth weight among female neonates. This study suggested significant negative associations between maternal urinary tOP concentrations and neonatal sizes at birth, and they differed by neonatal sex. Further epidemiological studies are required to more fully elaborate the associations between prenatal tOP exposure and birth outcomes.

PS 2964 Predicting Propofol Infusion Syndrome (PRIS): A Case-Control Study of Risk Factors and Outcomes

J. H. Diaz. *School of Public Health, Louisiana State University Health Sciences Center, New Orleans, LA.* Sponsor: M. Trimble.

Propofol (2, 6-diisopropylphenol), an intravenous sedative-hypnotic drug solubilized in soybean oil and egg phospholipid, is now used for intensive care unit (ICU) sedation in most cases. Propofol may rarely cause a fatal complication known as propofol infusion syndrome (PRIS). In order to identify predictive risk factors for PRIS, Internet search engines were queried to identify case reports of PRIS fatalities in ICU patients and to compare them with a similar control cohort of ICU patients also sedated with propofol, but not developing PRIS. 22 fatal case reports of PRIS were identified and compared with 1,006 no-PRIS control cases. Continuous variables were compared for differences by unpaired, 2-tailed t-tests; and categorical variables were compared by either X2 or Fisher exact tests with statistical significance defined by p-values < 0.05. PRIS patients were significantly younger (t=4.760, p<0.0001) than controls; received higher doses of propofol (t=6.657, p<0.0001) than controls; and were more likely to have neurological diagnoses (X2=10.089, p<0.001) including brain tumors, traumatic brain injuries, arteriovenous malformations, and status epilepticus. There were no differences in gender and in duration of propofol infusions between PRIS cases and controls. Metabolic acidosis, increased serum creatine kinase (CK>10,000 IU/L), increased serum potassium (K>5 mmol/L); and intravenous vasopressor use were significantly associated with PRIS fatalities. Serum hyperlipidemia, abnormal liver and renal function tests, and intravenous steroid use were not associated with PRIS fatalities. Young ICU patients with neurological diagnoses requiring larger doses of propofol for sedation and manifesting early increases in serum biomarkers of rhabdomyolysis, especially elevated CK and K, are at greater risks of PRIS. Although the molecular mechanisms of PRIS remain unknown and will require further investigation, individuals with underlying mitochondrial disorders may be at increased risks of PRIS due to an inability to transport exogenous lipids into mitochondria for energy generation during critical illnesses.

PS 2965 Toluene Diisocyanate and Formaldehyde Specific IgE and IgG Antibodies in Urethane Resin Handling Plant Workers

M. Tsuji[2], T. Isse[2], M. Yamamoto[1] and T. Kawamoto[2]. [1]*Basic Medical Sciences, National Institute for Minamata Disease, Minamata, Japan and* [2]*Envionmental Health, University of Occupational and Environmental Health, Kitakyusyu, Japan.*

Chemical substances are primarily used in industrial plants, where they may cause allergic symptoms in workers. While both IgE and IgG are related to allergies, only a few chemical allergens are targeted with the IgE screening tests used by companies in Japan. Furthermore, chemicals are often used in combinations, so it is difficult to estimate a single causal allergen based upon only disease history and questionnaires. In this study, we tried to clarify the following; 1. The relationship between toluene diisocyanate (TDI)- and formaldehyde (FA)-specific IgG and IgE antibodies. 2. The relationship between TDI- and FA-specific IgG, allergic diseases and exposure to TDI. The subjects were 77 male workers at a urethane resin plant in Fukuoka, Japan (Mean age 39.1 ± 1.2 years, the average length of service 11.5 ± 0. 8 years). We performed a questionnaire survey and collected blood samples in May 2013. TDI- and FA-specific IgG levels were measured by dot blotting. In addition, total IgE, total IgG, TDI- and FA-specific IgE levels were measured by CAP-FEIA. Chemical specific IgE-positive subjects were not necessarily chemical specific IgG antibody-positive. Chemical specific IgG-positive subjects had a history of allergy as compared to negative subjects. TDI workers had a tendency to be chemical specific IgG-positive as compared to non-TDI workers (OR 3.4; 95%CI 0.8-14; p=0.085), FA (OR 2.0; 95%CI 0.5-7.7; p=0.299). Along with TDI, FA was often used at this plant. It is not yet clear which immunoglobulin, IgE or IgG, is a better indicator of the chemical exposure or allergic onset because the number of Ig- positive subjects was very small. It will be necessary to increase the subject number in the future to address these important points. This study was funded with an Industrial Disease Clinical Research Grant (2014) and UOEH Research Grant for Promotion of Occupational Health (2012).

PS 2966 Records from the Swedish Poisons Information Center as a Means for Surveillance of Occupational Accidents and Incidents with Chemicals

L. Schenk[3], K. Feychting[1], A. Annas[1] and M. Öberg[2,3]. [1]*The Swedish Poisons Information Centre, Stockholm, Sweden;* [2]*The Swedish Toxicology Sciences Research Center (Swetox), Södertälje, Sweden and* [3]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

Official statistics of occupational injury and disease based on employers' incident reports are often incomplete. With the respect to chemical accidents and incidents at the workplace Poisons Information Centers (PIC) may serve as a supplement to official statistics. The present work is a retrospective review of five years of Swedish PIC records with the objective to describe the type of occupational accidents and incidents reported to the Swedish PIC (serves a population of about 10 million). The aim was to extract information that may be used for identifying preventive or mitigating measures. We extracted available information about occupational accidents in the Swedish PIC records from 2010 to 2014. We identified 8236 occupational incidents or accidents for which one or several calls have been made to the PIC. A majority of first calls are made by the public (n=5609), while about one-fifth of first calls are from physicians at hospitals (n=1286) or outpatient healthcare (n=327). Eye exposure is the most common exposure route recorded in our material (n=3049), followed by inhalation (n=2636) and skin (n=1425). In about 5% of cases the PIC records indicate several exposure routes. However, among calls from physicians 38% concern inhalation exposures and only 19% eye exposure. The three largest chemical groups involved in the incidents are alkali (n=1516, excluding ammonia), hydrocarbons (n= 1125, including halogenated hydrocarbons) and acids (n=971). Concerning the kind of products involved, different kinds of cleaning products are implicated in a substantial share of incidents (n=1750), mostly concerning eye or skin exposure and acids or alkali. One implication for risk management from these findings is that use of protective equipment should be promoted and rapid access to eye flush kits secured among employees that handle cleaning agents.

PS 2967 Effects of the Indoor Thermal Environment on Human Food Intake: A Pilot Randomized Controlled Trial

M. C. Bernhard[2], P. Li[2], D. B. Allison[2] and J. M. Gohlke[4]. [1]*Biostatistics, University of Alabama at Birmingham, Birmingham, AL;* [2]*Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL;* [3]*Nutrition Obesity Research Center, University of Alabama at Birmingham, Birmingham, AL and* [4]*Population Health Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Obesity etiology is thought to be multifactorial with contributors including genetic predispositions, behavioral factors, and environmental obesogens among many other factors. Model organism studies suggest environments with heightened ambient temperatures decreases food intake. We hypothesized that exposure to mild temperatures above the thermoneutral zone (TNZ) would have the potential benefit of decreased food intake in young adults in a sedentary office environment, as initially reported in Bernhard et al 2015 Front. Nutr. (2:20). Participants wearing standardized clothing were randomized to perform routine office work for 2 hr. in a TNZ environment, considered control (19-20°C), or an environment above the TNZ considered warmer (26-27°C) using a parallel-group design (n =11 and 9, respectively). Heat dissipation was estimated using peripheral temperature, from a thermal image using middle finger nail bed as a proxy. General linear models were built to examine the effects of thermal treatment on caloric intake and potential mediation by heat dissipation. The participants in the control conditions ate 99.5 kcal more than those in the warmer conditions; however, the difference was not statistically significant. After controlling for thermal treatment, gender and BMI, the participant's peripheral temperature was significantly associated with caloric intake (p = 0.002), suggesting a mediating effect. Specifically, for every 1°C increase in peripheral temperature suggesting increased heat dissipation, participants ate 85.9

kcal less food. This pilot study provided preliminary evidence suggesting the thermoregulatory mechanisms that increase blood flow to the periphery to increase heat dissipation may also act to decrease food intake. We are currently completing a study with a longer exposure period (7 hrs.) using a crossover design.

2968 Diet and Low Serum Vitamin D Are Associated with Increased Crisis-Related Hospitalization Rates Among Sickle Cell Disease Patients

M. McCaskill[2], O. Ogunsakin[2], H. Hottor[2] and R. Kruse-Jarres[1]. [1]*Puget Sound Blood Center, Seattle, WA and* [2]*Global Environmental Health Sciences, Tulane University, New Orleans, LA.*

Background: Sickle cell disease (SCD) is a genetic disorder resulting from the presence of a mutated hemoglobin S (HbS). Homozygous HbSS carriers will present with early manifestations of vaso-occlusive crises, which are characterized by body, bone, and joint pains. Environmental, dietary, and behavioral mediators may mediate vaso-occlusive crisis frequency. Vitamin D is a fat-soluble vitamin naturally present in very few foods, added to others, and available as a dietary supplement. The majority of people who suffer from SCD are chronically and severely deficient in vitamin D (<20 ng/ml). The overall objective of this study is to elucidate the complex relationship between vitamin-D serum levels, vitamin D containing food consumption and SCD-related hospital utilization. Methods: 1) A cohort of 109 SCD patients was administered a pain survey to determine the severity of pain experienced by patients during crises events. Patients were also asked to report level of sun exposure, and dietary intake of food containing vitamin D. 2) Vitamin D status and level of crisis-related ER/hospital utilization (over 12 months) was evaluated in 1500 de-identified Sickle Cell Clinic (SCCSL/SCD) patients. Results: Patients who reported to never consumed fish and milk in their diet had more hospital stay and ER visit when compared to those that consume these products on a daily basis. Those who consumed these products in their diet several times a month also had fewer ER visits when compared to "almost never" category. Ninety percent of surveyed SCCSL/SCA population had serum levels of vitamin D lower than 30 ng/ml. Patients with 25(OH)D3 levels less than 17 ng/ml have more crisis related hospital visits per year (10) than patients with 25(OH)D3 serum levels > 40 ng/ml. This increase in ER utilization is possibly due to diminished vitamin D-mediated immune functions among these patients, thereby increasing their susceptibility to infection and inflammation. Conclusion: Healthy serum vitamin D levels are directly associated with reduced hospital/ER visits. Additionally, diets that are high in fish, milk, cheese and eggs were also conditionally associated with reduced lengths of hospital/ER visits. Advanced studies must be conducted to elucidate the confounding effects of dietary, and environmental influences on crises severity in SCD patients.

2969 Dietary and Genetic Influence on the Metabolism and Biological Effects of Arsenic Exposure: A Double-Blind, Placebo-Controlled Folic Acid-Supplementation Trial

X. Chen[2], X. Yan[2], P. He[2], J. Zhu[2], J. Nie[2], D. Aga[2], H. Wu[3], X. Guo[3] and X. Ren[2]. [1]*Department of Chemistry, The State University of New York, Buffalo, NY;* [2]*Department of Epidemiology and Environmental Health, The State University of New York, Buffalo, NY and* [3]*School of Environment and Public Health, Wenzhou Medical University, Wenzhou, China.*

Arsenic, a naturally occurring element, is a world-wide public health concern affecting millions of people. Chronic arsenic exposure has been shown to be associated with increased morbidity and mortality from both cancerous and non-cancerous effects. To various degrees, studies have suggested that polymorphisms of enzymes involved in arsenic biomethylation, in particular arsenic (+3 oxidation state) methyltransferase (As3MT), and folate nutritional status may influence arsenic methylation therefore toxicity. However, very few studies have examined the combined effect of genetic polymorphisms of As3MT and folate nutritional status in arsenic metabolism. In addition, our recently identified new arsenic methyltransferase, N-6 adenine specific DNA methyltransferase 1 (N6AMT1), has not been fully characterized for the association between N6AMT1's genetic polymorphisms and arsenic biotransformation. To elucidate the dietary and genetic influence on the metabolism and biological effects of arsenic exposure, we conducted a randomized, double-blind, placebo-controlled folic acid-supplementation trial in a rural region of Inner Mongolia, China. Three hundred adults with arsenic exposure were enrolled in and randomly divided into three groups and given folic acid at a dose of 400 or 800 microgram/day, or placebo for 8 weeks. Plasma concentrations of folate and urinary concentrations of arsenic metabolites were analyzed at baseline and the end of trial. Genetic polymorphisms of As3MT and N6AMT1 were obtained using Agena iPLex genotyping methods. While data is currently under the analysis, preliminary data indicates that folic acid supplementation enhances arsenic methylation and polymorphisms of As3MT and N6AMT1 influence arsenic metabolisms and may modify folate's effects. Moreover, our data suggests that folate supplement may modify arsenic-induced alteration of global DNA methylation, thus may be used as a preventive measure to reduce the risk of arsenic-related health outcomes. (This work was supported by NIEHS grant R21ES022329 and R01ES022629 to X.R.)

2970 Spatial Analysis of Environmental Contaminants Found Historically in a Polycythemia Vera Cancer Cluster Area

K. Benson, A. Ragin-Wilson, J. S. Wheeler and E. Irvin-Barnwell. *CDC/ATSDR, Atlanta, GA.*

In 2004, the Pennsylvania Department of Health identified an area in northeast Pennsylvania with a higher than expected number of cases of a rare cancer called polycythemia vera (PV) and requested the assistance of the Agency for Toxic Substances and Disease Registry (ATSDR) to further investigate. ATSDR confirmed 33 cases of PV in the tri-county area and identified a statistically significant cluster of 15 PV cases at the nexus of three counties: Luzerne, Carbon, and Schuylkill. The purpose of this study is to conduct an ecologic analysis using environmental data for contaminants historically present in the tri-county area to investigate potential clustering. Environmental data for the tri-county area were obtained from a data warehouse created as part of the ATSDR PV cancer cluster investigation. The data warehouse incorporates information from 47 facility site assessments and 16 online sources and includes approximately 12,300 water sampling records for the 11 chemicals included in our analysis, arsenic, benzene, benzo(a)pyrene, chloride, chromium, phenol, radium226, tetrachloroethylene (PCE), trichloroethylene (TCE), perfluorooctanoic acid (PFOA), and perfluorooctane sulfonate (PFOS). The spatial analysis includes the boundaries of the original cluster area and a 10-mile buffer zone, including residential locations for 40 confirmed PV cases, and 9 EPA superfund sites. Using the Getis-Ord Gi* Statistic in GIS, hot spot analyses were performed for each chemical to identify any significant clustering around the PV cluster area and 10-mile buffer zone. GIS was used to compare the distribution of chemicals geographically in the three counties, aggregately and over 5 year intervals. Preliminary analyses indicate potential clustering inside the buffer zone around the original cluster area for all chemicals except PFOA, PFOS, and benzo(a)pyrene. Additionally, potential clustering outside the buffer zone was indicated for arsenic, chloride, and chromium. Future plans include analyzing environmental data across other media, including soil, and to investigate if environmental contamination clustering occurred near the PV case residential locations.

2971 Genetic and Epigenetic Differences by Tobacco Smoking and Quitting Smoking

M. Yang. *College of Pharmacy, Sookmyung Women's University, Seoul, Korea, Republic of.* Sponsor: M. Yang.

Considering health risks of tobacco smoking, many governments have grappled with tobacco addiction and tried to prepare proper (personalized) strategies for quitting smoking. For this purpose, we studied genetic and epigenetic alteration by smoking and quitting smoking. We performed whole genome analyses with expression and methylation chips (N=12 of men smokers and nonsmokers). As results, we found that the DNA-methylation of CpG sites was clearly altered by smoking, rather than the gene expression. When the criteria of DNA methylation, such as 'number of CpG islands at a gene', 'delta, i.e. difference in the DNA methylation (%) between two groups, >0.1', 'the location at CpG islands', etc., were considered, the DNA methylation status at BCYRN1, INGX, BTNL2, MIR886, GSTM1, SLC17A9, DUSP22, etc. were different due to smoking. In a case of DUSP22, which was reported to be hypomethylated in fire fighters, we studied association between smoking and its DNA methylation levels by methylation specific PCR and found somewhat (≈10%) hypomethylation of the DUSP22 by smoking among the above chip subjects, although it was not significant (p>0.05). In a trial of quitting smoking with bupropion (N=27), some genetic polymorphisms, e.g. VAV3, ACTG1 and KCNJ2/SOX9, showed association with smoking cessation. Therefore, we report potential of epigenetic alteration by tobacco smoking and genetic variations in quitting smoking. This study was supported by the National Research Foundation of Korea (NRF) funded by the Korea government (MEST) (2011-0030702).

2972 Acrolein Metabolites and Diabetes and Insulin Resistance

F. Scinicariello[3], A. G. Feroe[2] and R. Attanasio[1]. [1]*Department of Biology, ATSDR, Georgia State University, GA;* [2]*Department of Biology, Bowdoin College, Brunswick, ME and* [3]*Division of Toxicology and Human Health Sciences, ATSDR, Atlanta, GA.* Sponsor: M. Mumtaz.

Background: Acrolein is a ubiquitous pollutant. Acrolein protein adducts have been detected in people with diabetes and diabetic complications. Acrolein has been associated with dysregulation of glucose transport in endothelial cells. Objective: To examine the association of urinary acrolein metabolites S-(3-hydroxypropyl)-N-acetylcysteine (3-HPMA) and 2-carboxyethylmercapturic acid (CEMA) with diabetes and diabetes marker risk such as insulin resistance as a categorical outcome expressed by the homeostatic model assessment (HOMA-IR>2.6), HOMA-IR, beta-cell function (HOMA-β), fasting insulin and fasting plasma glucose. Methods: We performed analyses of adults who participated in the 2005-2006 National Health and Nutrition Examination Survey. We performed multivariate logistic regression to analyze the association of diabetes and insulin resistance (after exclusion of participants taking insulin or other diabetes medications) with concentrations of single urinary acrolein metabolites and their molecular mass sum (∑acrolein). Furthermore, we performed multivariate linear regression with HOMA-IR, HOMA- β, fasting insulin and fasting plasma glucose as continuous dependent variables. Results: Diabetes as well as insulin resistance (defined as HOMA-IR>2.6) were positively associated with the 3-HPMA, CEMA and ∑acrolein with evidence of a dose-response trend ($p < 0.05$). The highest 3rd and 4th quartiles of CEMA compared to the lowest quartile were associated with higher HOMA-IR, HOMA- β and fasting insulin with a dose-response relationship. The highest 3rd quartile of 3-HPMA and ∑acrolein were positively and significantly associated with HOMA-IR, HOMA-β and fasting insulin. Conclusions: This is the first study reporting an association between urinary acrolein metabolites and diabetes marker risk in a large nationally representative survey in adults 20 and older. Urinary acrolein metabolites were associated with diabetes and insulin resistance.

2973 Sodium Fluoride Induces Apoptosis in H9c2 Cardiomyocytes by Altering Mitochondrial Membrane Potential and Intracellular ROS Level

X. Yan[2], Y. Qiu[2] and J. Wang[1]. [1]*Animal Science Department, Shanxi Agricultural University, Taigu, China and* [2]*Health Public School, Shanxi Medical University, Taiyuan, China.* Sponsor: X. Ren.

Background: Chronic excessive fluoride intake is known to be toxic, and effects of long-term fluorosis on different organ systems have been examined. Studies showed that fluoride has severe detrimental effects on cardiac function in atherosclerosisrelated myocardial injury and pathophysiological remodeling. However, very few studies have ever addressed the mechanism of action of fluorosis in cardiovascular system. Here, we studied the fluoride-induced apoptosis in H9c2 cells and determined the underlying molecular mechanisms including the cell viability, intracellular reactive oxygen species (ROS) level, the changes of mitochondrial membrane potential (ΔΨm), and the cell apoptosis. Methods and Results: Sodium fluoride (NaF) at concentrations of 0, 2, 4, 8, and 16 mg/L was administered to cultured H9c2 cells for up to 48 h. After the treatment, H9c2 cells were collected and the associated parameters were measured by flow cytometry. Compared with the control group, numbers of viable cells were moderately increased at low concentrations but decreased with high NaF concentration. Our results demonstrate that NaF upregulates the intracellular ROS level in H9c2 and down-regulated the ΔΨm in NaF-induced H9c2 cells. For apoptosis analysis, NaF treatment induces both early and late stage cell apoptosis in a dose-dependent fashion. At the end of 48 h, the total apoptotic cells were increased by 23.16 and 28.27 % in the presence of 2 and16 mg/L NaF, respectively. Conclusions: Our study found that fluoride not only inhibited H9c2 cell proliferation but also induced cell apoptosis. With the increment of NaF concentration, the apoptotic rates and ROS generation were increased, while the ΔΨm was decreased. In summary, these data suggested that NaF-induced H9c2 cell apoptosis is mediated by direct increased intracellular ROS and downregulated ΔΨm.

2974 Asbestos Exposure and Pharyngeal and Laryngeal Cancer Risk: A Fiber-Type Specific Meta-Analysis

S. Benson[2], S. Batdorf[2], K. Hitchcock[2], D. Galbraith[3] and B. Finley[1]. [1]*Cardno ChemRisk, Brooklyn, NY;* [2]*Cardno ChemRisk, Pittsburgh, PA and* [3]*Cardno ChemRisk, San Francisco, CA.*

In 2012, The International Agency for Research on Cancer (IARC) concluded that asbestos exposure was associated with an increased risk of laryngeal and pharyngeal cancers. The degree to which different fiber types (i.e., chrysotile vs amphiboles) might possess different potencies for these cancer endpoints was not evaluated. This meta-analysis investigated the association between laryngeal and pharyngeal cancers in occupational cohorts exposed to either amphibole, chrysotile, or mixed (both chrysotile and amphibole) fibers. We considered all of the studies evaluated by IARC, as well as many others. A total of 37 studies were included in our analyses, and the cohorts included workers in mining, textile, cement, insulation, and other industries. Various fiber type designations were considered. The first designation evaluated the fiber exposures as indicated by authors of the manuscript. If only chrysotile or only amphibole fiber types were mentioned, those studies were considered to be chrysotile and amphibole specific exposures. Mixed fiber was indicated if the study mentioned potential exposures to chrysotile with any amphibole fiber, regardless of their individual proportions. The second method employed designations as indicated in previous studies that evaluated "predominantly chrysotile" fiber cohorts. Consistent with the findings of IARC, our analyses of "all fiber types" exposures (chrysotile, amphibole, and mixed fibers treated as a single dataset) indicated significant associations with laryngeal and pharyngeal cancers, with meta-RRs of 1.47 (95% CI: 1.29, 1.68) and 1.32 (95% CI: 1.07, 1.62), respectively. Results by fiber type were consistent with pooled analyses for amphibole exposures demonstrating the highest significant effect estimates. The strength of the association decreased with mixed and chrysotile exposures, but the significance of the effect was dependent upon the classification of fiber type. Overall, the results indicate a trend in fiber potency which is consistent with fiber potency differences known to exist for lung cancer and pleural mesothelioma.

2975 Quantitative Bias Analysis of a Reported Association between Perfluorooctanoic Acid (PFOA) and Endometriosis

G. Ngueta[2,4], M. Yoon[1], H. Clewell[1,3], M. E. Andersen[1,3], M. P. Longnecker[3] and M.-A. Verner[2,4]. [1]*Hamner Institutes for Health Sciences, Durham, NC;* [2]*Public Health Research Institute (IRSPUM), University of Montreal, Montreal, QC, Canada;* [3]*Ramboll Environ, Durham, NC and* [4]*Public Health Research Institute, Department of Occupational and Environmental Health, University of Montreal, Montreal, QC, Canada.*

A recent epidemiological study reported an association between endometriosis and plasma PFOA levels (Louis et al. 2012). Women with endometriosis experience more pain during menses (dysmenorrhea) than controls and are therefore more likely to use oral contraceptives (OCs) as a treatment for pain. Reduction of menstrual blood loss by OCs (70%) could decrease PFOA elimination and confound the association between PFOA and endometriosis. We estimated the influence of OC use for dysmenorrhea on the association between plasma PFOA and endometriosis. We used a one-compartment pharmacokinetic model of plasma PFOA and a Monte Carlo process to simulate 20,000 women like those in the epidemiological study. The probability of disease (case/control) and dysmenorrhea (yes/no) for each woman was determined based on published distributions. In women with dysmenorrhea, we evaluated different probabilities of taking OCs to treat pain (0%-100%). Duration of OC treatment for pain was randomly sampled (0.5-10 years before diagnosis). The relation between endometriosis and simulated plasma PFOA levels (ng/ml) was evaluated using logistic regression. We observed greater odds ratios with increasing probabilities of women using OCs to treat dysmenorrhea. When assuming that 100% of women with dysmenorrhea used OCs, the odds ratio was 1.08 (95% CI=0.98-1.18). In comparison, the odds ratio in the epidemiological study, which was not adjusted for OC use, was 1.62 (95% CI=0.99-2.66). Future plans include consideration of different probabilities of OC use for dysmenorrhea, depending on disease status. Under the assumptions made, our results suggest that confounding by OC use may explain some of the epidemiological association between endometriosis and plasma PFOA levels.

2976 Meta-Analysis of Lung Cancer Risk Related to Diesel Exposure by Occupation and Evaluation of Exposure Response

J. Panko, S. Benson and M. Kreider. *Cardno ChemRisk, Pittsburgh, PA.*

In 2012, the International Agency for Research on Cancer (IARC) changed the designation of diesel exhaust from a probable to causal lung carcinogen. Their decision was based on a qualitative assessment of many cohorts that had diesel exposure but also could have had exposure to other lung carcinogens. They did not attempt to account for any potential co-exposures nor did they evaluate whether there was a dose response relationship for diesel exposure and cancer risk. Our objective was to conduct meta-analyses for three occupational cohorts that are often overlooked in the evaluation of diesel exhaust exposure and the risk of lung cancer: dock workers, heavy equipment operators, and diesel engine mechanics. In all, 17 peer-reviewed manuscripts (n = 7 for dockworkers, n = 8 for heavy equipment operators, and n = 9 for mechanics) met our inclusion criteria and specifically identified diesel exhaust as the exposure of interest. Pooled effect estimates (meta-RR) and their corresponding 95% confidence intervals (CI) were calculated for each occupational group using the DerSimonian and Laird random effects model. Heavy equipment operators (meta-RR = 1.38; 95% CI: 1.00, 1.89) and dock workers (meta-RR = 1.34; 1.07, 1.68) had comparable risks of lung cancer, but mechanics were not at an increased risk (meta-RR = 1.15; 95% CI: 0.93, 1.44). Using exposure estimates reported in the IARC monograph we calculated the median elemental carbon exposures from diesel emissions for dock workers (7 µg/m^3), diesel engine mechanics (20 µg/m^3), and heavy equipment operators (47.5 µg/m^3). In short, the diesel exposure estimates do not correspond to the risk estimates, which suggests the lack of a dose-response relationship. The increased risks observed could be related to exposures to other carcinogens or other confounding factors. Hence, a finding of "risk" for any given diesel cohort may not necessarily mean the risk was related to diesel exhaust.

2977 Placental Cadmium Levels are Associated with Increased Preeclampsia Risk

J. E. Laine, P. Ray, W. Bodnar, P. H. Cable, K. Boggess, S. Offenbacher and R. C. Fry. *University of North Carolina, Chapel Hill, Chapel Hill, NC.*

Environmental exposure to heavy metals is a potentially modifiable risk factor for preeclampsia (PE). Toxicologically, there are known interactions between the toxic metal cadmium (Cd) and essential metals such as selenium (Se) and zinc (Zn), as these metals can protect against the toxicity of Cd. As they relate to preeclampsia, the interaction between Cd and these essential metals is unknown. The aims of the present study were to measure placental levels of Cd, Se, and Zn in a cohort of 172 pregnant women from across the southeast US and to examine associations of metals levels with the odds of PE in a nested case-control design. Logistic regressions were performed to assess odds ratios (OR) for PE with exposure to Cd controlling for confounders, as well as interactive models with Se or Zn. The mean placental Cd level was 3.6 ng/g, ranging from 0.52 to 14.5 ng/g. There was an increased odds ratio for PE in relationship to placental levels of Cd (OR = 1.5; 95% CI: 1.1-2.2). The Cd-associated OR for PE increased when analyzed in relationship to lower placental Se levels (OR = 2.0; 95% CI: 1.1-3.5) and decreased with higher placental Se levels (OR = 0.98; 95% CI: 0.5-1.9). Similarly, under conditions of lower placental Zn, the Cd-associated OR for PE was elevated (OR = 1.8; 95% CI: 0.8-3.9), whereas with higher placental Zn it was reduced (OR = 1.3; 95% CI: 0.8-2.0). Data from this study suggest that essential metals may play an important role in reducing the odds of Cd-associated preeclampsia and that replication in a larger cohort is warranted.

2978 Lung Cancer Mutation Profile: Meta-Analysis and Comparison of Never and Ever Smokers

A. M. Chapman[1], K. Y. Sun[1], P. Ruestow[2], D. M. Cowan[1] and A. K. Madl[1]. [1]*Cardno Chem Risk, Aliso Viejo, CA and* [2]*Cardno Chem Risk, Chicago, IL.*

Lung cancer is the leading cause of cancer-related mortality. While the majority of lung cancers are associated with tobacco smoke, approximately 10-15% of U.S. lung cancers occur in never smokers. Evidence suggests that lung cancer in never smokers appears to be a distinct disease with separate genetic pathways caused by driver mutations, unlike the widespread genomic changes observed in lung cancer of smokers. A meta-analysis of human epidemiologic data was conducted to evaluate the profile of common or therapy-targetable mutations in lung cancers of never and ever smokers. Epidemiologic studies (N=174) representing over 63,000 lung cancer cases were identified and used to calculate summary odds ratios for lung cancer in never and ever smokers containing gene mutations: EGFR, chromosomal rearrangements and fusion of EML4 and ALK, and KRAS. This analysis also considered the effect of histopathology, smoking status, sex, and ethnicity. Results showed there were significantly increased odds of presenting the EGFR and ALK-EML4 mutations in 1) adenocarcinomas compared to non-small cell lung cancer and 2) never smokers compared to ever smokers. The prevalence of EGFR mutations was higher in Asian women as compared to women of Caucasian/Other ethnicity. As the smoking history increased, there was a decreased odds for exhibiting the EGFR mutation, particularly for cases >30 pack-years. Compared to ever smokers, never smokers had a decreased odds of KRAS mutations among those of Caucasian/Other ethnicity (OR = 0.22, 95% CI: 0.17-0.29) and those of Asian ethnicity (OR = 0.39, 95% CI: 0.30-0.50). Available epidemiologic data suggest that environmental or occupational factors (e.g., asbestos) may not be associated with the unique mutation profile of EGFR or KRAS in lung cancers of never or ever smokers, respectively. Our findings show that key driver mutations and several patient features are highly prevalent in lung cancers of never smokers, which may provide useful information as targeted treatment modalities are developed for lung cancer.

2979 Does Longevity Play an Etiological Role in Pleural Mesothelioma?

B. S. Beckerman[3], E. T. Chang[2], G. Mezei[2] and B. D. Kerger[1]. [1]*Exponent, Irvine, CA;* [2]*Exponent, Menlo Park, CA and* [3]*Exponent, Oakland, CA.*

We hypothesize that a fraction of incident mesotheliomas spontaneously arise and may be related to increased longevity. Using the US SEER 9 cancer registries (1973–2012), we investigated gender-specific time trends in age-adjusted incidence rates of pleural mesothelioma. Rates were age-standardized to the US 2000 population. We used Social Security lifetables to estimate denominators for ages 85+ at individual age-years to enable detailed adjustment for age-related confounding. We compared rates in males and females 0-74 and 75+ years. We estimated mesothelioma incidence trends estimated using a degree-one spline with one knot, and. 95% confidence intervals (CI) using bootstrap sampling. Coefficients are presented in cases per 100,000 person-years. In males 0–74, rates increased, with beta 0.012 (CI 0.0036, 0.055) until 1991 (CI 1980, 1994); rates then decreased, with beta -0.022 (CI -0.027, -0.012) to 2012. In females 0-74, rates initially appeared flat, with beta 0.0023 (CI -0.0003, 0.0054) until 1990 (CI 1987, 2002), when rates decreased, with beta -0.0032 (CI -0.0060, -0.0023). Based on mean trends in the 0-74-year age group, male and female rates would intersect in 2024 (CI 2021, 2056). In males 75+ years, rates initially increased, with beta 0.557 (CI 0.464, 0.775) until the end of 1992 (CI 1987, 1995); rates then flattened, with beta -0.054 (CI -0.169, 0.046). Females 75+ years showed no significant trend; this result was sensitive to one outlier in 1974. Without the outlier, rates initially increased, with beta 0.085 (CI 0.045, 0.135) until 1984 (CI 1982, 2006); rates then flattened, with beta 0.034 (CI -0.035, 0.043). For older men and women, rates showed similar patterns, but absolute rates in men were much higher. The declines at ages 0-74 are presumably related to asbestos exposure, whereas the flat trends at ages 75+ may be due to an opposing effect of the aging population. In summary, while causal associations are not asserted, the results suggest increased longevity may result in greater incidence of pleural mesothelioma unrelated to asbestos.

2980 An Analysis of Toxic Chemical Releases and Cancer Rates in Louisiana Parishes

S. Ponnapakkam, C. Rothenberg and E. Svendsen. *Global Environmental Health Sciences, Tulane University, New Orleans, LA.* Sponsor: J. Wickliffe.

Citizens of Calcasieu Parish have been concerned that the large amounts of factories in the area have had an adverse effect on their health. This study serves to examine the chemical releases and cancer rates of African American men and women in Calcasieu Parish in comparison to Louisiana Parishes and US counties from 2001–2010. Calcasieu Parish had higher chemical releases of Polycyclic Aromatic Hydrocarbons in comparison to Louisiana Parishes ($p<0.01$) and US counties ($p<0.01$) and higher malignant neoplasm rates of respiratory and intrathoracic organs compared to the US ($p<0.01$) and Louisiana Parishes ($p=0.059$), but no statistical correlation was revealed between exposures and health outcomes when confounding variables were controlled for, implying that future studies should look to control for more confounding variables. Future studies should also look to examine more spatially-oriented data in order to observe whether releases were near highly populated areas such as schools.

2981 Metal Biomonitoring in the Navajo Birth Cohort Study: Relationship to Uranium Mine Waste and Other Exposure Sources

J. Lewis, J. Hoover, J. Ong and C. Shuey. *Community Environmental Health Program, University of New Mexico, Albuquerque, NM; Southwest Research and Information Center, University of New Mexico, Albuquerque, NM.*

The Navajo Birth Cohort Study (NBCS) is a prospective birth cohort study examining the relationships among ongoing exposure to atomic bomb and Cold War Era legacy wastes from >500 abandoned uranium mines on Navajo Nation (NN), birth outcomes, and child development. Although the last mines closed in 1986, >1100 associated waste sites remain in communities. The study is being conducted as a cooperative agreement with CDC/ATSDR, Navajo Area IHS, Navajo Department of Health, Southwest Research and Information Center, and University of New Mexico's Community Environmental Health Program. Of the target of 1500 families, 568 mothers, 406 babies, and ~30% of fathers are currently enrolled in the study. Blood and urine samples are collected at multiple time points from enrollment until the child is one year of age, and analyzed for 36 metals at CDC/ATSDR/DLS/IRAT laboratories. Home environmental assessments are conducted measuring indoor home dust, indoor and outdoor gamma radiation, indoor radon, and drinking water quality. Surveys complete the exposure profile through queries on land-use activities, occupational and health history, and nutritional information. Results to date show that the distribution of urine uranium in NBCS adults is shifted to the right of the US population (NHANES, 2015), with >17% of the participants with urine uranium concentrations exceeding the 95th%ile seen in US adults. Of babies with complete results, 0.7% exceed the 95th%ile for US adults at birth, increasing to 10.9% at 6 months, and 30.8% at 1 year. Concentrations of manganese, inorganic and total mercury, antimony, tin and tungsten also show greater concentrations than expected relative to NHANES. Surprisingly, total arsenic, a common contaminant in unregulated water and mine waste throughout NN, is seen at lower concentrations than for the US population, as is lead. Iodine deficiency is common, and many in the population show zinc deficiency. Home environmental assessments indicate >80% of homes have detectable uranium in the dust, while >60% of homes show other metals exceeding indoor dust health-based screening levels, including lead, arsenic, manganese, iron, antimony, and aluminum. Source attribution is in progress, as are analyses to model exposure against birth and developmental outcomes.

2982 Undergraduate Field Study of Community Arsenic Exposure in Rural Bangladesh

Z. Paquette, C. Stone and J. Angermann. *School of Community Health Sciences, University of Nevada, Reno, NV.* Sponsor: J. Angermann.

More than nine million tubewells provide drinking water to 97% of Bangladesh's rural population. The WHO recommends a 10 µg/L limit for inorganic arsenic in drinking water, yet the Bangladeshi government has set a standard of 50 µg/L for practical reasons. In December 2013 and 2014, University of Nevada Community Health Science undergraduate students enrolled in a senior field study course studied household tubewell arsenic trends in Naria Upazila of the Shariatpur district of Bangladesh. The field study focused on determination of inorganic arsenic in drinking water, enumeration of health effects associated with chronic arsenic exposure, and remediation of residential water supplies with high arsenic loads. The goal was for the undergraduate researchers to demonstrate professional and ethical behavior while conducting service learning activities with their matched Bengali peer health professionals. Students learned and applied methods for clinical assessment of the health effects of arsenic exposure and were responsible for collecting water samples from each tubewell for arsenic determination. At each location students worked with translators to administer a cross-sectional retrospective cohort survey, as well as record coordinates using handheld global positioning systems to characterize geospatial trends in arsenic distribution. In total 161 surveys were administered and 156 tubewells were sampled, yielding arsenic levels up to 183 µg/L. As an intervention to reduce arsenic exposure, 'Kanchan' filters were built, which utilize iron oxide chelation and depth filtration to reduce arsenic in supplied water. Using local supplies, students were able to successfully construct and deliver seventeen of these filters to families consuming the highest concentrations of arsenic detected in the region. Through this field study project, students were able to apply many aspects of their undergraduate public health curriculum to the development of innovative and effective solutions to a pressing international public health issue.

2983 Gestational Week-Specific Curve for Small-for-Gestational Age (SGA) Risk Attributable to Maternal Tobacco Use

H. Ferdosi[1], J. Polifka[3], J. Li[2] and S. Lamm[1]. [1]*Center for Epidemiology and Environmental Health, CEOH, LLC., Washington, DC;* [2]*Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD and* [3]*Department of Pediatrics, University of Washington School of Medicine, Seattle, WA.*

Background: While small for gestational age (SGA) is a well-known consequence of maternal smoking, the magnitude of risk by week of gestational age has not been elucidated. To assess that risk, we analyzed the 1990-2002 birth certificate data for central Appalachian states. Methods: Singleton live births (N = 3,032,928) with birth weight, gestational age (22-44 weeks), and maternal tobacco use history were categorized as SGA or not, based on 10th percentile gender-specific weights-for-age. SGA prevalence was analyzed for tobacco users and non-users, yielding relative risks and odds ratios. Gestational week-specific rates, rate differences, and multivariate logistic adjusted odds ratios were also analyzed. Results: SGA prevalences among tobacco users (19.5%) and non-users (9.1%) yielded a significant SGA prevalence rate ratio and a significant odds ratio of 2.15 and 2.43, respectively. The pattern for SGA by gestational week was similar for comparative rates, rate differences, and adjusted odds rates. The rate for tobacco non-users was steadily near 9% across the gestational age range of 22 to 44 weeks. The rate for tobacco users was steady until week 33 when it rose monotonically through week 37 to about 20% at week 38 and remained high. Tobacco use was not seen to be an SGA risk factor for births of gestational age of 33 weeks or earlier. The magnitude of tobacco use as an SGA risk factor for third-trimester births increased during the period of premature birth and became fully evident with a two-fold risk for full term infants. Conclusion: We newly report the temporal pattern of tobacco-related SGA by week of gestational age. Tobacco-related SGA was only seen for third trimester births - increasing during weeks 33 through 37 with a doubling during weeks 38–44. This pattern is informative for issues of mechanism and highlights the potential benefit of extending tobacco cessation programs through the third trimester of pregnancy.

2984 Role of Metals and Aspects of Socioeconomic Status (SES) in Peripheral Arterial Disease in the US Population

A.-R. M. Kadry, Y.-S. Lin, D. Segal and B. Sonawane. *National Center for Environmental Assessment, US EPA, Washington, DC.*

Atherosclerosis and atherothrombosis are serious pathological changes and are responsible for various disease conditions such as peripheral arterial disease (PAD). The prevalence of PAD, commonly assessed by reduction of Ankle-Brachial Index (ABI), is over 10% in the US population over 60 years old. The data from the Atherosclerosis Risk in Communities (ARIC) Study indicated that the prevalence of Lower Extremity Arterial Disease (Lower Extremely PAD) in non-Hispanic Blacks is higher than that of non-Hispanic Whites. While there is evidence indicating that both metals and non-chemical stressors (e.g., SES) play a role in PAD risk, the findings remain largely inconclusive. The goal of the study is to test the hypothesis that both chemical exposure and SES play a role in PAD by examining the relationship of SES (assessed by income, size of house hold, number of household residents, PIR and BMI), race, and chemical stressors (lead, cadmium, and iron) with PAD risk using a population-based, nationally representative sample from the National Health and Nutrition Examination Survey (NHANES, 1999-2000). The analysis was restricted to non-Hispanic Whites, non-Hispanic Blacks, and Mexican-American participants aged 20 years and up with valid ABI measurements and variables of interest (e.g., blood levels of metals and SES). The analysis results suggest that the ABI generally decreases with increasing levels of lead and cadmium, but with decreasing blood iron. Lower ABI also tended to be more prevalent among subjects with lower SES (e.g., annual household income < $20,000). Of note, ABI is also considerably lower among non-Hispanic Blacks (mean ± SE: 1.08 ± 0.01), as compared to non-Hispanic Whites (1.14 ± 0.01) and Mexican Americans (1.15 ± 0.01) ($p < 0.001$). The decline in ABI was most evident among non-Hispanic Blacks with lower SES as compared to affluent non-Hispanic Blacks or other races regardless of SES. Additional studies are warranted to examine cumulative and/or synergistic effects of chemical stressors and SES as well as race on PAD.

2985 Pathway Analysis of Prenatal Exposure to Phthalates and Child Motor Development

D. A. Sarigiannis[2,4], K. Polanska[3], G. Theodoridis[2] and C. Chatziioannou[2]. [1]*Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [3]*Environmental Epidemiology, Nofer Institute of Occupational Medicine, Lodz, Poland and* [4]*Environmental Health Engineering, Institute of Advanced Study, Pavia, Italy.* Sponsor: T. Hartung.

Phthalate prenatal exposure was determined by targeted metabolomics measuring 11 phthalate metabolites in urinary samples from (n=165) mothers during the third trimester of pregnancy (prenatal exposure) and from their children at the 24th month of age (postnatal exposure), using HPLC-MS/MS. Psychomotor development was assessed in children at the age of 2 years by the Bayley Scales of Infant and Toddler Development. Child motor development was inversely associated with natural log concentrations (µg/g creatinine) of 3OH-MnBP (β = - 2.3; 95% CI - 4.0 to - 0.6), 5OH-MEHP (β = - 1.2; 95% CI - 2.2 to - 0.3), 5oxo-MEHP (β = - 1.8; 95% CI - 3.3 to - 0.2) and sum of DEHP metabolites (β = - 2.2; 95% CI - 3.6 to - 0.8), DnBP metabolites (β = - 1.9; 95% CI - 3.4 to - 0.4), and high molecular weight phthalates (β = - 2.5; 95% CI - 4.1 to - 0.9) in the urine collected from mothers during pregnancy after adjustment for a variety of potential confounders. Postnatal child exposure to phthalates was not associated with any of the measured scores of child psychomotor development. Further untargeted metabolomics analysis (using a 600 MHz NMR) of the mother urine samples, revealed that the mothers with higher exposure to phthalates have completely different metabolic profiles compared to the ones with lower exposure levels. Metabolic pathway analysis using Agilent GeneSpring revealed that alterations in urine metabolites are related to the TCA cycle, suggesting impaired mitochondrial respiration; the latter is central to energy metabolism and cellular signaling and plays fundamental roles in synthesis of nucleotides and active transport processes. Inhibition of mitochondrial oxidative phosphorylation could also cause a defective mitochondrial energy production during the process of fetus formation and development. These fundamental biological pathway perturbations are reflected in early life motor development.

2986 Legacy of Lead Water Service Lines: Hazard Assessment, Exposure Modeling and Implications to Public Health, Policy and Outreach

A. Katner, C.-y. Hu and J. Diaz. *Environmental and Occupational Health Sciences, LA State University Health Sciences Center, New Orleans, LA.* Sponsor: C. Miller.

Drinking water is presumed to be a minor lead source, but our data indicate utilities may be underestimating water lead levels. We evaluated New Orleans drinking water lead levels (WLLs) using refined sampling and processing procedures, which demonstrated improved lead recovery. Samples were collected to evaluate the US Environmental Protection Agency's (EPA) water utility compliance testing guidelines (first draw samples) and prevailing exposure prevention guidelines (30 sec to 2 minute flush prior to use) (n=242 samples from 64 homes). The EPA's Integrated Uptake Biokinetic Model was used to estimate the impact of WLLs on childhood blood lead levels. Water lead levels ranged from <1 to 220 µg/L (mean=4.8 µg/L; median= 1.4 µg/L). Eleven percent of sites sampled exceeded the EPA Action Level (15 µg/L), thus New Orleans drinking water did not meet regulatory requirements, contrary to utility results. Over half of our tested homes had WLLs exceeding health-based standards (56% exceeded Cal-EPA's Public Health Goal and 80% exceeded EPA's Maximum Contaminant Level Goal). Ninety-two percent of homes had WLLs resulting in childhood blood lead levels exceeding the European Union's reference value (1.2 µg/dL) based on site-specific WLLs and model default lead levels in soil, air, dust and diet. Of homes we sampled with both first draw and flushed samples (n=60 homes), 52% had increased WLLs after flushing for 30 seconds to 2 minutes, indicating that first draw samples collected for utility compliance may not result in lead detection, despite presence in subsequent draws; and that prevailing guidelines to flush water for 30 seconds to 2 minutes may be inadvertently increasing lead exposures. Due to ineffective lead recovery practices, utilities may not be taking regulatory action in situations that warrant it. EPA's Lead and Copper Rule should be revised to optimize utility compliance testing requirements; and lead poisoning prevention messages should be revised to protect public health, especially in cities with aging water infrastructure.

2987 Limitations of Regulatory Tier I and II Approaches to Demonstrate Equivalence of Generic Biocides

T. Wako[2], K. Ozaki[2] and J. Pauluhn[1]. [1]*Bayer CropSience, Monheim, Germany and* [2]*Environmental Health Division, Sumitomo Chemical Co., Ltd, Tokyo, Japan.* Sponsor: J. Pauluhn.

Requirements of bioequivalence and relevant impurities are in place for the evaluation and registration of new compounds with biocidal or pesticidal activities. However, ambiguity exists as to how 'equivalence of health hazards' can unequivocally be demonstrated by analytical (Tier I) and toxicological (Tier II) assays. The case presented in this analysis focuses on the chiral pyrethroid transfluthrin (TFL) and the chemically reactive impurity permethrinic acid anhydride (PAA). This study compared reference TFL with generic TFLs from two alternative manufacturing sources in India and China. Most of the Tier I analyses yielded higher levels of PAA with variable purity and impurity levels from one batch to another. Most of the alternative source TFLs were biologically less effective. Tier II studies provided evidence of lung irritation and extreme skin sensitization in specialized bioassays, i.e., the Local Lymph Node (LLNA) and the GPMT assays. Sensitized guinea pigs challenged with PAA-protein conjugate aerosol responded positively. Regulatory limits on skin sensitizers tend towards 10 ppm (0.001%) where the sensitizer is extreme, exposure is uncontrolled, and in long duration regular contact. For PAA, being an extreme sensitizer, a regulatory-driven specific concentration limit (SCL) of 0.001% was applied for skin sensitization. Due to the additional potential to induce respiratory sensitization the SCL is lowered by one third. This SCL is about 1/100 of the Benchmark-NOAEL-95% of the LLNA. The concentration of TFL used in household insecticides (evaporators) commonly does not exceed 1%. Assuming an upper specification limit of PAA in TFL of 0.03%, the SCL of any typical formulation is set at 0.0003%. Based on this paradigm and the fact that PAA is both a skin and respiratory sensitizer, 0.03% PAA in TFL technical grade is considered to be the SCL. Collectively, 'structural alert'-based Tier II tests proved to be more predictive than any indiscriminant battery of standard bioassays commonly applied or default Tier I approaches.

2988 The Read-Across Assessment Framework Under REACH

N. Andersson, D. R. Bell, I. Bichlmaier, G. Cartlidge, K. De Raat, N. Fedtke, A. Kojo, A. Kovari, T. Netzeva and E. Stilgenbauer. *Evaluation, European Chemicals Agency (ECHA), Helsinki, Finland.* Sponsor: K. Savolainen.

Grouping of substances and read-across approach is an adaptation of the standard testing regime set out in Annex XI, Section 1.5 of the REACH Regulation (EC No 1907/2006); this adaptation allows registrants, under certain conditions, to predict the properties of a substance based on data from structurally similar substances. To facilitate the evaluation of read-across cases encountered in the different REACH processes, ECHA has developed a read-across assessment framework (RAAF). The first version of the RAAF is focussed on human health. It is a scientific framework which structures the knowledge that is relevant for evaluating a read-across case. The framework directs and guides the assessing expert by posing relevant questions and suggesting possible answers. The RAAF defines different scenarios for different read-across approaches (e.g. two structurally similar compounds are assumed to have the same effect(s); or the prediction is based on a trend for the same effect(s) observed within a category). The respective scenarios are selected and applied to the proposed cases. Each scenario is associated with particular aspects (assessment elements, AEs) that are deemed crucial to the assessment (e.g. are the same effect(s) observed for the substances). Each AE poses questions which lead an assessing expert to select pre-defined conclusions (assessment options, AOs). The AOs reflect the strengths and weaknesses of the read-across for the aspects covered by the AEs (e.g. 'Acceptable with medium confidence'; or 'Not acceptable in its current form'). The outcome is a degree of confidence associated with the proposed read-across. In summary, ECHA has developed a RAAF, which structures expert judgement, so that the criteria for expert opinions on which regulatory decisions are based can be applied consistently. The RAAF increases transparency on how ECHA assesses read-across cases and provides registrants with a focus to assess and improve their cases.

2989 Surrogate Evaluation and Read Across for Hazard Assessment: Best Practices and Potential Pitfalls

T. A. Lewandowski and J. M. Cohen. *Gradient, Seattle, WA.*

Due to increased requirements for chemical hazard assessment, read across analyses have become commonplace in toxicology. The available computational tools and approaches for conducting read across analyses vary considerably in terms of sophistication, ease of use, and comprehensiveness, leading to read across analyses of inconsistent quality. Two key elements of a read across analysis involve quantifying structural similarity and predicting common mode of action (e.g., via structural alerts). To better understand the utility of different analytical tools in these areas we established a set of 67 chemicals (e.g., aniline, benzocaine, dinitrophenol, ethylenediamine) with structural similarity to a target chemical (phenylenediamine). All chemicals in the set have published animal test data on skin sensitization. We first evaluated chemical similarity using two computational tools (Toxmatch and ChemMine). We found that the two programs often gave different quantitative estimates of chemical similarity. However, when chemicals were ranked according to their similarity score relative to the target chemical of concern, the two rank lists demonstrated statistically significant agreement (Kendall's Coefficient of Concordance, W = 0.85, p = 0.0003). We also evaluated the data set for structural alerts for dermal sensitization using the Structure Activity Relationship (SAR) program Toxtree and compared the SAR predictions to animal test data. Toxtree had a sensitivity of 78.4% (correctly predicting 29 out of 37 animal test positives), and a specificity of 80.6% (identifying 25 out of 31 animal test negatives). Surrogate selection accuracy was improved when physico-chemical data were used to identify suitable surrogates for read across analysis in addition to structural alert analysis. Our analysis shows that selection of suitable surrogate chemicals for read across requires careful application of available SAR tools and data on chemical properties to improve the validity of the safety assessment.

2990 Retrospective Analysis of Acute Toxicity Tests for Pesticides

M. Paris[2], J. Strickland[2], D. Allen[2] and W. Casey[2]. [1]*NIEHS/NICEATM, Durham, NC and* [2]*ILS, Inc., NIEHS/NICEATM, Durham, NC.*

Systemic toxicity from exposure to toxic chemicals, chemical products, and pharmaceuticals is a significant cause of illness and death in the United States. U.S. regulatory agencies use data from *in vivo* rodent acute oral and dermal toxicity tests to determine potential systemic toxicity of chemical products following ingestion and topical skin exposure. These data are used to derive an LD50 value (dose expected to produce lethality in 50% of the animals tested) for hazard classification and labeling to protect human health and the environment when handling and transporting chemicals. In this study, we considered whether rat acute oral toxicity LD50 values for pesticide formulations and active ingredients (AIs) could be used to determine acute dermal toxicity hazard classifications for the U.S. Environmental Protection Agency (EPA) categorization system and the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). This retrospective analysis used high-quality acute toxicity data for 910 formulations and AIs from various EPA toxicity reports, peer-reviewed publications, and databases. Oral hazard classifications based on rat oral LD50 values were compared to dermal hazard classifications based on rat dermal LD50 values. The concordance of oral and dermal hazard classification was 52% for dermal hazard using the EPA system and 65% for dermal hazard using the GHS system. Using oral hazard classifications overestimated dermal hazard for 37% of the compounds using EPA classifications and 34% using GHS classifications. Predictivity of classification for substances that do not require hazard labeling (EPA Category IV [LD50 >5000 mg/kg] and GHS unclassified [>2000 mg/kg]), was 73% for the EPA system and 99% for the GHS system. Underprediction of dermal hazard was 11% for the EPA system and 2% for the GHS system, which indicates that acute oral hazard categories are sufficiently protective for acute dermal hazard classification. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

2991 Optimal Sampling and Extraction Procedures of Ergot Alkaloid Analysis

T. Grusie, V. Cowan, J. McKinnon and B. Blakley. *University of Saskatchewan, Saskatoon, SK, Canada.* Sponsor: L. Weber.

Ergot, a mycotoxin produced by the fungus *C. purpurea*, is a contaminant of cereal crops and grasses. Current food and feed safety guidelines allow food and feed grain to contain 0.01-0.05% and 0.10% net ergot weight, respectively. Variation at low concentrations can lead to unreliable estimates of ergot concentration which can expose humans and animals to toxic levels. Therefore, a reliable and sensitive chemical analytical method to detect low-levels of ergot alkaloids is essential to ensure food and feed safety. This study was conducted to assess how the grinding method, sample volume and sample concentration affect the variability of total ergot alkaloid concentration during extraction and liquid chromatography - mass spectrometry (LC-MS) analysis. The grinding method was assessed comparing two grinders (coffee grinder and sample mill) during the extraction process of samples containing one ergot body. The sample volume and concentration were assessed by preparing five concentrations, ranging from 0.002% to 5% net ergot weight, at four volumes (75, 250, 500 and 1000 mL). All samples were ground then extracted using an extraction solvent (85% acetonitrile / 15% 10 mM ammonium acetate) and analysed via LC-MS. Grinding method revealed that it can exceed a 10-fold difference in standard deviation demonstrating substantial variability. At diagnostically relevant ergot concentrations smaller sample volumes also exhibited greater variability. The coefficient of variation was approximately 7 times greater at relevant food safety concentrations as compared to that of the highest ergot concentration evaluated. Extreme variation compromises interpretation from a food safety perspective. To conclude, this study demonstrated that grinding method and smaller sample volumes exhibit significant variability, particularly at low concentrations. A standardized grinding method at a minimum 1000 mL sample volume should be used to reduce unreliable ergot concentration estimates.

2992 Dose Response Curves Derived From Clinical Ozone Exposures Can Inform Public Policy

S. S. Lange[2], G. Tao[1], L. R. Rhomberg[1], J. E. Goodman[1], M. L. Dourson[3] and M. E. Honeycutt[2]. [1]*Gradient, Cambridge, MA;* [2]*Toxicology, Texas Commission on Environmental Quality, Austin, TX and* [3]*Toxicology Excellence for Risk Assessment Center, University of Cincinnati, Cincinnati, OH.*

Ozone is one of six criteria air pollutants for which regulations are set by the US Environmental Protection Agency using the National Ambient Air Quality Standards (NAAQS). The ozone NAAQS level was recently changed to 70 parts per billion (ppb) from 75 ppb, but debate is ongoing about the public health protection afforded by this change. Our study used data from human clinical studies to inform policy decisions about a protective ozone level. We plotted mean forced expiratory volume (FEV1) response versus total inhaled ozone dose (calculated from ozone concentration, duration of exposure and ventilation rate) from clinical studies of 1–8 hour durations. There were clear thresholds of effect, which were consistent with the known ozone mode of action. The initial plot used data from healthy young adults; additional analyses incorporated data from children and asthmatics, but results did not differ. We estimated expected ozone doses that would be experienced by the population based on known exercise ventilation rates and durations, at ambient ozone concentrations of 75, 70 or 65 ppb (8-hour maximum average). Doses were similar at these three concentrations, and most doses were below those associated with a mean 5% FEV1 decrement, even when different exposure times and ventilation rates were assessed. This type of analysis can determine thresholds of ozone toxicity, which can be crucial for choosing an ambient ozone concentration that is protective of human health.

2993 Exploring the Potential Use of Reach Inhalation Derived No Effect Levels (DNELs) for Workers as a Source of Information to Update OSHA PELs

M. P. Jackson[2], L. Fell[1,2] and R. Lemus[1]. [1]*Advanced Testing Laboratory, Cincinnati, OH and* [2]*Haley & Aldrich, Inc., Ann Arbor, MI.*

The last significant effort by the US Occupational Safety and Health Administration (OSHA) to update the Permissible Exposure Levels (PELs) occurred over 25 years ago. Because many of the PELs have not been updated despite the availability of new data, in October 2014, OSHA issued a request for information regarding its current approach of managing chemical exposures in the workplace, and considers the European

Union's Regulation on the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) as one of the information sources to use for occupational exposure assessments and the PELs update process. We have compared DNELs and PELs for several metals (n=11) and volatile organic compounds (VOCs) (n=22) as an initial step to evaluate the information value of the European Chemical Agency (ECHA) database that contains more than 10,000 chemicals. The DNEL is defined as "the level of exposure above which humans should not be exposed" and needs to reflect the likely route(s), duration and frequency of exposure. Specifically, worker DNELs are levels intended to protect workers during manufacture and downstream uses. Our preliminary results showed that for metals, 18% of PELs were lower, 18% of PELs were the same, and 64% of the PELs were higher than the respective DNELs. Similarly, for VOCs, 3.5% of PELs were lower, 18% of the PELs were the same, and 78.5% of the PELs were higher than the respective DNELs. Overall, this brief analysis suggests that reviewing the industry's submitted REACH data present in the ECHA database is worth exploring to support either a decrease/increase in the current occupational exposure limit, or to confirm that the current OSHA PEL is still protective after being established several years ago.

2994 Common Misconception: Rats Receive a 4–5 Times Lower Ozone Tissue Dose Than Humans at the Same Concentration

D. D. McCant, J. T. Haney, S. Lange and M. E. Honeycutt.
Toxicology, Texas Commission on Environmental Quality, Austin, TX.

Ozone (O3) has a NAAQS of 70 ppb. Typically, regulatory concentrations are extrapolated from animal toxicity data in the absence and/or along with human data. Based on Hatch et al. (1994), researchers often are under the misconception that human tissue doses of O3 should be expected to be 4-5 times greater than rat doses at equal exposure concentrations, leading to the incorrect assumption that rats must be exposed to 4-5 times a given human exposure concentration to achieve the same O3 dose. However, this is a misconception as Hatch et al. did not expose humans and rats under the same conditions. The study exposed exercising humans to 0.4 ppm and resting rats to 2 ppm and found comparable incorporation of 18O into the bronchoalveolar lavage constituents. This important difference in activity state has not always been appropriately considered or accounted for when the perceived implications of the Hatch et al. study are cited in the peer-reviewed literature. However, this difference in activity state explains the comparable incorporation of 18O by exercising humans and resting rats at 5-fold different exposure concentrations. More specifically, although exercising humans were exposed to a 5-times lower O3 concentration than resting rats, their inhalation rate was 5-times higher than that at resting, offsetting the 5-times lower exposure concentration and producing the same dose that would be expected at rest when exposed to 2 ppm (i.e., 0.4 ppm x exercising human study inhalation rate of 64.6 L/min ≈ 2 ppm x resting human study inhalation rate of 13.5 L/min). In other words, humans exposed to 2 ppm at rest should be expected to experience the same dose as exercising humans at 0.4 ppm, which produced a dose comparable to resting rats at 2 ppm. Correcting the misconception in the literature that rats must be exposed to 4-5 times environmental concentrations to achieve the same environmentally-relevant O3 dose as in humans is important for scientists and policy makers who are making regulatory decisions.

2995 A Computational Approach to Evaluate NIEHS-Funded Scientific Literature in the Context of the 2012–2017 NIEHS Strategic Plan

K. J. Chandler, E. Milliman, L. S. Birnbaum and S. A. Newton.
National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NIEHS launched a strategic plan in 2012, "Advancing Science, Improving Health: A Plan for Environmental Health Research", to serve as a framework for the field of environmental health sciences. Multidisciplinary themes and goals were woven throughout this plan with an emphasis on the importance of scientific research in achieving success across the integrated themes and goals. We hypothesized that text mining, topic modeling, and visualization tools applied across the NIEHS-funded scientific literature curated in PubMed may serve as a method to begin evaluating implementation of the strategic plan. 23,878 NIEHS-funded scientific titles and abstracts published between fiscal year (FY) 2009-2015 were obtained from PubMed in XML format and used to build a corpus for subsequent text analysis and topic modeling in R. Term frequency-inverse document frequencies were calculated to assess the importance of terms across the corpus to build word clouds for each FY and FY quarter. Machine learning algorithms were used to build models of 25, 50, 100, 250, 500, and 1000-topics from the corpus and the topic model structure was visualized using DendroArcs. These analyses have allowed us to begin examining how topic emphasis and relationships have evolved over time. Global comparisons of the 50-topic model between FY 2009 and 2015 reveal more extensive connections between most topics in FY 2015. The average number of connections between topics in FY 2015 was 12, as compared to nine in FY 2009, with the greatest number of connections to the "pathway, biology, function, molecular" topic in both fiscal years (2009=20; 2015=26). Moving forward, text mining and computational approaches may aid evaluation of strategic plan implementation and could be expanded beyond PubMed abstracts. This research was supported by the NIH, National Institute of Environmental Health Sciences, Division of Intramural Research and Office of the Director.

2996 Exposure of Regulated Drinking Water Disinfection Byproducts and Existing Compliance

S. Parvez[2], K. Frost[2] and M. Sundararajan[2]. [1]*Environmental Epidemiology, Indiana University Fairbanks School of Public Health, Indianapolis, IN and* [2]*Environmental Health, Indiana University Fairbanks School of Public Health, Indianapolis, IN.*

Disinfected drinking water is daily consumed by billions of people worldwide. Disinfection byproducts (DBPs) are the unintended formation products of water disinfection and suspected to cause cancer, reproductive toxicity, and developmental health problems. Stage 2 DBP compliance was established to control the maximum concentration levels (MCL) of the two most widely reported DBPs, 4-Trihalomethanes (THMs) and 5-Haloacetic acids (HAAs) by tightening compliance monitoring. Despite further tightening, exposure scientists and epidemiologists believe compliance data is inadequate to capture true population exposure. To understand this ambiguity, we designed a residential tap water monitoring study for determining differences in residential exposure levels and compliance data. We collected weekly and biweekly water samples simultaneously from different residences (total 8 sites), relying on a major community water system. This distribution system consist of five treatment stations, each gets water from a separate source water body. All treatment stations use chloramine as their primary disinfectant. All participants across the distribution system were strategically recruited to capture different residential locations and treatment stations. A total of 30 samples were collected during a three month period (May through August). The collected samples were analyzed within 7 days of collection by an EPA certified lab using EPA's standard methods. Our comparison of monthly mean concentrations during the peak DBP season with annual LRAA data indicates compliance data underestimate the exposure in peak months. The residents rely on surface water treatment stations show significantly higher concentrations for THMs and HAAs with 27% of samples exceeding the MCL for THM4 and 35% exceeding the HAA5 MCL. Although, more work is needed to comprehensively study this in other water systems, our findings indicate that water compliance needs to be developed using residential data rather than system-wide data to capture representative human exposure.

2997 Alberta Environmental Public Health Information Network

N. Wang[2], E. Ellehoj[1], J. Mayhew[3] and W. Kindzierski[3]. [1]*Ellehoj Redmond Consulting, Edmonton, AB, Canada;* [2]*Health Protection Branch, Alberta Health, Edmonton, AB, Canada and* [3]*School of Public Health, University of Alberta, Edmonton, AB, Canada.*

There is a recognized need to develop and improve the availability of health information for decision-making across numerous fields, communities, professions, organizations, institutions, and health systems. In Alberta, there is professional and public interest in having access to information and data related to health status and other factors about the Alberta population. An environmental public health network integrates information and data on health, environmental hazards and exposure that is intended to drive actions to improve the health of communities and to support human health risk assessment. Alberta Health and the University of Alberta are developing a network called the Alberta Environmental Public Health Information Network (AEPHIN). AEPHIN is an internet-based public health information system that offers user-friendly and interactive presentations of various types of environmental and public health data for Alberta and its population. Initially, there will be three environmental health datasets: 1) domestic well water quality, 2) contaminants in local foods (mercury in fish), and 3) cyanobacteria levels in recreational water bodies. It also presents public health data on demographics, socioeconomics, and a measure of deprivation and it links to historical statistics (e.g., incidence and prevalence rates) on mortality, chronic and infectious disease, and children's health in Alberta. AEPHIN can be used to inform and support policy develop-

ment and actions related to public health and serve as an education and communication tool for Albertans regarding public health and environmental health-related information. Finally, the network can serve as a platform for researchers to access data, formulate their own hypotheses and conduct further research accordingly.

2998 Toxicity, Exposure, and Fate of Acrylonitrile in Communities - A Risk Management Perspective

A. Pawlisz and D. Johnson. *GHD, Dallas, TX.*

Industrial chemicals in the environment may have various origins, from accidental releases to permitted discharges. Public health concerns arise when there is limited understanding of actual exposures, chemical characteristics, and toxicity, especially during environmental emergencies when multiple, often conflicting risk communication messages are provided by the general and/or social media. Several authors have commented on the lack of timely expert risk statements in readily-accessible media. Instead, general public typically obtains risk/hazard avoidance information from non-expert sources. The objective of the presentation is to demonstrate how the lack of expert statements on toxicity, exposure, and fate may trigger community concerns. This demonstration is based on a hypothetical release of acrylonitrile (AN). Peer-reviewed publications, public databases, subscription databases, and government databases were searched for information related to physicochemical properties, environmental fate, toxicological effects, and health criteria of AN. Next, risk communication publications were reviewed to identify the most common concerns when spills occur in communities. The combined results were compared and contrasted to draw conclusions regarding the perceived vs. the potential for actual risks. Results show that spill-impacted communities are most frequently concerned with air and water quality, safety of food and garden produce, and household items left indoor/outdoor. From the actual exposure perspective, AN is a water soluble liquid so it may be found in recreational and potable water sources, though its residence is expected to be brief (days). AN has a moderately high vapor pressure and may evaporate to be inhaled by receptors. Absorption through skin is possible, but evacuations and exclusion perimeters remove residents from the direct contact pathway. AN has low organic carbon:water (Koc) and octanol:water (Kow) partition coefficients so it is not likely to adsorb to soil, sediment, and common surfaces, or bioaccumulate in foods. While acute exposures lead to convulsions and coma, and in most species, death appears to be related to metabolite (cyanide) poisoning, chronic exposures are more common in communities and are generally well tolerated. In summary, communities near spill are prone to react with specific health concerns, which may not have actual risks basis. Therefore, early and pro-active risk communication by experts is needed to avert the unnecessary stress on communities.

2999 "Pressured to Prescribe" Impact of Economic and Regulatory Factors on ED Physicians When Managing Drug Seeking Patients

S. Kelley, G. Johnson, J. Wolfson and R. Harbison. *Environmental Occupational Health, College of Public Health, Tampa, FL.*

The ED (emergency department) physician faces unique challenges in changing healthcare and economic climates. Requirements to address pain as the "fifth vital sign", patient satisfaction based reimbursement metrics, and an economically driven rise in ED patient volume, may have inadvertently created an environment conducive to exploitation by prescription opioid abusers. There is an identified need for the development of continuing medical education (CME) and standardized regulatory and legislative protocols to assist ED physicians in the appropriate management of patients exhibiting "drug seeking" behavior. The purpose of this study was to elicit the opinions of ED physicians, currently practicing in the United States, regarding the impact of economic and regulatory factors on their management of patients exhibiting "drug seeking" behavior. A descriptive, cross-sectional study, utilizing a convenience sample of ED physicians was conducted for a period of two months. The inclusion criteria specified that any ED physician, currently practicing within the United States, could participate. Of the ED physicians surveyed (n=141), 71% reported a perceived pressure to prescribe opioid analgesics to avoid administrative and regulatory criticism and 98% related patient satisfaction scores as being too highly emphasized by reimbursement entities as a means of evaluating their patient management. Rising patient volumes and changes in the healthcare climate were cited by ED physicians as impacting their management of patients exhibiting "drug seeking" behavior. The results of this study have identified several gaps in training, regulation, and administrative healthcare practices that are potentially deterring efforts of ED physicians in their management of patients with "drug seeking" behavior. Further research is needed to identify appropriate interventions to bridge these identified gaps.

3000 Assessment of Retractions as a Measure of Scientific Misconduct

A. Abritis, G. Johnson and R. Harbison. *Environmental Occupational Health, College of Public Health, Tampa, FL.*

Fraud or fabrication in research has caused loss of time, funds, and in some cases, lives. Those attempting to analyze research misconduct have commonly utilized counts of articles retracted for misconduct as a measure of the depth and breadth of the problem. However, the retraction of an article occurs so far downstream in the research publication process that it is unlikely to be a suitable variable. Therefore, it is hypothesized that the use of retractions for misconduct will not accurately represent the prevalence of research misconduct. To evaluate this hypothesis, finalized misconduct findings from the Office of Research Integrity from 1993 to 2013 were reviewed to determine the number of published articles related to the misconduct that were retracted, corrected or otherwise amended. Furthermore, published notices of these types of amendments were examined for the assigned cause of the amendment. Of the 167 subjects with finalized findings, 84 (50.3%) had no associated published article while 72 (43.1%) had 1 or more articles retracted for misconduct. 174 retraction notices were located, with less than half (46.0%) of the notices attributed to misconduct. More than half of the retractions resulting from research misconduct indicated error or some other reason for the cause of retraction. Additionally, 13 articles specifically referenced in the final findings had no associated retraction or correction located in the databases. Pearson correlation coefficients for the relationships between retractions indicating misconduct and total combined retractions, total corrections and retractions, and total retractions and corrections indicating misconduct as cause were 0.4549, 0.3465, and 0.3969, all far lower than would be expected in light of the confirmation of misconduct. Thus, the use of retractions is inappropriate for quantifying the degree of misconduct within scientific research, and can mislead researchers into underestimating the prevalence of misconduct.

3001 Environmentally-Induced Epigenetic Toxicity and Public Health: The Next Challenge for Regulatory Toxicology

E. L. Marczylo, T. W. Gant and M. N. Jacobs. *Toxicology, Public Health England, Chilton, United Kingdom.*

Epigenetic changes regulate gene expression without mutating DNA and can be modified by environmental chemicals. To determine whether, or not, environmentally-induced epigenetic toxicity is of real concern to public health there are some important considerations, including dose, route, metabolism, and timing of exposure; mixture effects; human variability; adaptive versus adverse outcomes; and mechanisms and model systems. To date there is no precedent for regulating epigenetically-mediated toxicity. However, such toxicity continues to be of high concern for all OECD member countries, particularly with respect to developmental, later life adverse health and multigenerational risks. Research studies investigating mechanisms of epigenetic toxicity have already helped to identify putative biomarkers and assays that may be useful for biomonitoring such exposures and toxicities, and testing the epigenetic toxicity potential of environmental exposures. In addition, current OECD human health related Test Guidelines (TGs) have the potential for adaptation to incorporate adverse epigenetic endpoints that would begin to address regulatory concerns and develop formal epigenetic toxicity TGs. Using our own and others' published data on cigarette smoke, phthalates and estradiol, we show how TG 483 could be adapted by further utilising spermatozoal samples. The analysis of spermatozoal RNAs, species known to be both susceptible to perturbation by environmental exposures and result in phenotypic changes, could add a valuable epigenetic component to this new genotoxicity TG. Such TG adaptations have a far-reaching positive impact on the regulatory community, enabling improved cost- and time-effective, and scientifically-valid assessments. Regulations are a fine balance between maximising the vast benefits of a chemical whilst minimising any potential adverse effects. Thus, it is better for both society and public health to fully assess the potential epigenetic effects of environmental exposures and continue to further modify TGs and regulations based on relevant data and proven mechanisms of toxicity.

3002 Vanadium–What Should Constitute a "Do Not Drink" Warning?

L. J. Bradley. *Haley & Aldrich, Northbridge, MA.*

Vanadium has been in the news in North Carolina (NC) due to concerns over coal ash contamination and its presence in groundwater, as evidenced by sampling of private wells conducted by the State. The wells have been sampled under the NC Coal Ash Management Act, which requires that all private wells within a 1,500-foot radius of a coal ash impoundment be tested for a variety of inorganic constituents, including vanadium. Almost 90% of the wells tested, including wells identified by NC as background wells that could not have been impacted by ash impoundments, have been issued a "Do Not Drink" letter from the State. The majority of these letters are based on a drinking water screening level of 0.3 ug/L for vanadium (or 0.6 ug/day for a 70 kg adult consuming 2L water/day). The basis of the derivation is not available. The NC screening level for vanadium is inconsistent with the current U.S. Environmental Protection Agency Regional Screening Level for vanadium in tap water of 86 ug/L (or 172 ug/day), and the Institute of Medicine's Tolerable Upper Intake Level of 1,800 ug/day for vanadium, which is defined as "the highest level of daily nutrient intake that is likely to pose no risk of adverse health effect for almost all individuals." For context, most adult daily vitamins provide 10 ug vanadium per day, and the U.S. adult diet contains up to 20 ug vanadium per day. The NC screening level for vanadium in drinking water is the lowest in the U.S. Data available for municipal water supplies in NC indicate that some do not meet the NC screening level for vanadium. Detailed independent hydrogeologic analyses of the coal ash ponds in NC indicate that groundwater from these areas is not moving off site, thus not impacting private wells, indicating that the vanadium in the private wells is naturally occurring, or from another source. The very low drinking water screening level for vanadium used by NC and consequent exceedances of that screening level have led to understandable fear and confusion on the part of the public in NC. This perhaps could have been avoided if a more complete evaluation of vanadium in the context of state background levels and levels established by other authoritative agencies had been thoroughly reviewed before adopting the standard.

3003 Regulatory Acceptance of Non-Standard Toxicological Methods through Increased Use of Integrated Approaches to Testing and Assessment (IATA)

C. Willett. *The Humane Society of the US, Washington, DC.*

There are scientific, economic, practical and regulatory pressures stimulating the development and use of streamlined chemical testing and assessment approaches, including an increased reliance on non-standard methods. Advances in biological understanding as well as experimental technologies (e.g. 'omics tools, stem cell culturing, reconstructed tissues), have allowed the consideration of dramatically different approaches to understanding disease and toxicology than those traditionally practiced, and increasingly, collections or batteries of non-testing and non-animal test methods are starting to replace apical animal tests. Articulation of the underlying perturbations of biology, for example in the form of adverse outcome pathways (AOPs) that link molecular initiating events to key intermediate events to the adverse outcome, can be used to form the logical basis for the integration of tests that query key events in a pathway. The use of an AOP-based testing strategy within the regulatory decision context, in the form of an IATA, could form the basis of facilitated regulatory acceptance. Assessment of reliability and performance the individual assays and prediction tools is first carried out using fundamental assessment principles derived from US, EU and OECD guidance. Results of these assessments would be considered during evaluation of the IATA for regulatory applicability - it is the IATA, using the non-standard methods that is reviewed and accepted by regulatory authorities. The use of high-throughput tests to evaluate estrogen receptor activity by the US EPA to address their endocrine program will be discussed of one potential example of how such an approach could be used to facilitate acceptance of non-standard methods more generally. In light of number and variety of new methods and types of information that could be used to better inform regulatory decisions, new faster, more efficient approaches are needed to support both assay assessment and harmonized regulatory use; the proposed approach is one possible solution.

3004 Safety of Carotenoids from Fermentation Using *Yarrowia lipolytica*

J. A. Edwards, P. Beilstein and J. Schierle. *DSM Nutritional Products Ltd, Kaiseraugst, Switzerland.* Sponsor: A. Thiel.

High purity crystalline zeaxanthin produced by fermentation from *Yarrowia lipolytica* has been tested for safety, in a similar way to β-carotene from *Yarrowia* (Grenfell-Lee et al., 2014). Crystalline zeaxanthin from Yarrowia, after extraction and purification, fulfils the >96% (3R,3'R)-zeaxanthin purity specification of our synthetic zeaxanthin. Crystalline zeaxanthin from *Yarrowia* was prepared in DMSO and tested in an Ames test (Bacterial Reverse Mutation Assay, OECD 471) using histidine-requiring strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* and tryptophan-requiring strain WP2 uvrA pKM101 of *Escherichia coli*. In a 13-week rat dietary study (OECD 408), a beadlet formulation containing 5% zeaxanthin was tested at dosages up to 500 mg zeaxanthin /kg bw/day. The beadlet matrix was the same as used for commercial synthetic zeaxanthin. The upper dosage was restricted to avoid a potential nutritional impact of the high content of the beadlets in the diet. A comparative control of beadlets with synthetic zeaxanthin (high dosage only) was also included. The two studies were undertaken at Covance Laboratories Ltd, UK, under GLP conditions. The Ames test, with concentrations up to the solubility limit in the solvent, was negative in all strains tested. In the 13-week rat study, 500 mg zeaxanthin /kg bw/day did not cause any effect considered to be adverse. The systemic exposure as measured by total zeaxanthin in the plasma was closely similar for synthetic zeaxanthin and zeaxanthin from *Yarrowia*. The two new studies presented here show very similar results to the corresponding two safety studies previously undertaken with synthetic zeaxanthin. The new studies confirm the safety profile of another high purity, crystalline carotenoid derived from *Yarrowia lipolytica*. Grenfell-Lee D et al., The safety of β-carotene from *Yarrowia lipolytica*, Food and Chemical Toxicology, Volume 65, March 2014, 1-11

3005 Comparative Evaluation of Liver Findings in Safety Studies with Canthaxanthin

J. A. Edwards[1], P. Beilstein[1] and L. Mecklenburg[2]. [1]*DSM Nutritional Products Ltd, Kaiseraugst, Switzerland and* [2]*Safety Assessment and Pathology, Covance Preclinical Services GmbH, Münster, Germany.* Sponsor: A. Thiel.

Canthaxanthin is a natural carotenoid and approved as a feed additive. Its safety has been extensively tested in various laboratory animals. These studies included a gavage study in cynomolgus monkeys that extended up to 5 years in duration with dosages up to 1000 mg/kg bw/day. Summarized data for this study is generally available in an EFSA re-evaluation in 2010. In this evaluation, the monkey liver pathology data is reviewed and compared with liver findings from corresponding rat, mouse and dog studies. Plasma level and liver exposure data from the monkey study were re-evaluated on a gender-specific basis. An original rat carcinogenicity study with dosages from 250 to 1000 mg/kg bw/day revealed a small increase in benign liver cell tumors in females without a clear dosage dependency. Supplementary 2-year studies carried out focusing on liver effects and investigating lower dosages (5 to 250 mg/kg bw/day) showed no treatment-related increase in liver neoplasia and the NOAELs defined by the authors were 5 and 25 mg/kg bw/day in females and males, respectively. Histological liver effects were associated with increases in incidence of hepatocyte enlargement, liver weights, and serum enzyme activities. The effect on hepatocellular tumors was not confirmed in mice. Neither a long-term toxicity study in dogs (52 weeks) nor the study in cynomolgus monkeys (up to 5 years) revealed any evidence for pre-neoplastic or neoplastic findings in the liver. Plasma and liver exposure data from monkeys obtained after two years of treatment increased with dosage but with evidence of saturated absorption at high dosages. The concentration curves were similar in both genders. The absence of liver toxicity or gender difference in liver response to chronic canthaxanthin treatment in primates is consistent with the data in the mouse and dog. The primate data adds to the evidence that for canthaxanthin the higher sensitivity to liver toxicity in the rat, in particular females, is species specific.

3006 Protective Effect of Oxides Nanoparticle Against Aflatoxicosis in Rats

S. Samir Essa[1], E. El-Saied[2], M. Mahmoud[1] and O. El-Tawil[2]. [1]*Immune Section, Research Institute for Animal Reproduction, Giza/Cairo, Egypt and* [2]*Toxicology Department, Cairo University, Faculty of Veterinary Medicine, Giza/Cairo, Egypt.*

This study was conducted to determine the efficacy of nano-composite Magnesium Oxide and Silicon Oxide (MgO-SiO2) to reduce the toxic effects of aflatoxin B1 (AFB1) exposure in adult male rats for 8 weeks. Animals were randomly divided into a control and three experimental groups (25 each). The first experimental group received feed contained 200 ppb AFB1. The second group received feed contained 200ppb AFB1/kg and 0.5 g/kg nano-composite of MgO-SiO2. Rats of the third experimental group received 0.5 g/kg nano-composite of MgO-SiO2. Result showed that AFB1 markedly reduced body weight gain and food and water consumption. Postmortem examination reveled that AFB1 intake also induced pathologic changes in the liver and spleen. Activities of lymphocyte transformation and serum lysozymes showed significant changes. On the other hand, the deleterious effects of AFB1 were alleviated in the group that received feed contain 200ppb of AFB1 plus 0.5g/kg MgO-SiO2. These findings suggested that MgO-SiO2 has high affinity to adsorb AFB1 and can effectively modulate its toxicity in rats. In addition, the nano-composite of MgO-SiO2 may offer a novel approach to the preventive management of aflatoxicosis in animals.

3007 Toxicological Assessment of Nattokinase Derived from *Bacillus subtilis* var. *natto*

B. Lampe and J. C. English. *NSF International, Ann Arbor, MI.*

Subtilisin NAT, commonly known as "nattokinase," is a fibrinolytic enzyme produced by the bacterial strain *Bacillus subtilis* var. *natto*, which plays a central role in the fermentation of soybeans into the popular Japanese food natto. Recent studies have reported on the potential anticoagulatory and antihypertensive effects of nattokinase administration in humans, and with increased interest in the potential health benefits of nattokinase, it is important to develop a comprehensive safety profile. In order to characterize the risk of adverse health effects associated with human consumption, several *in vitro* and *in vivo* assays in rodents were conducted with the nattokinase enzyme product NSK-SD (supplied by Japan Bio Science Laboratory Co., Ltd., Japan), including: an *in vitro* bacterial reverse mutation assay in standard Salmonella and *E. coli* strains; a chromosome aberration test in a CHL/IU fibroblast cell line; oral gavage studies in Sprague-Dawley rats, including a single dose assay and two repeated dose assays (treatment durations of 28 days and 90 days, respectively); and a pathogenicity test in mice inoculated with single doses of NSK-SD producing bacteria (7.55 x 108 CFU). Nattokinase was non-mutagenic in bacterial cells and non-clastogenic in mammalian cells. No adverse effects were observed in Sprague-Dawley rats given single doses of NSK-SD up to 2000 mg/kg, or repeated doses of 167 mg/kg-day and 1000 mg/kg-day with durations of 28-day and 90-day, respectively. Mice inoculated with 7.55 x 108 CFU of the enzyme-producing bacterial strain showed no signs of toxicity or residual tissue concentrations of viable bacteria. The animal data are consistent with clinical studies in humans, in which volunteers who ingest daily doses of 35 - 40 mg/day NSK-SD for 4 weeks experience no adverse health effects other than occasional instances of constipation or diarrhea. These findings suggest that the oral consumption of nattokinase is of low toxicological concern, with a subchronic NOAEL of 1000 mg/kg-day in rats.

3008 *In Vivo* Toxicity of Moniliformin

M. Jonsson[1], J. Atosuo[3], M. Jestoi[1], A. Nathanail[1], U.-M. Kokkonen[1], M. Anttila[1], A. Welling[1], P. Koivisto[1], E.-M. Lilius[3] and K. Peltonen[2]. [1]*Finnish Food Safety Authority, Helsinki, Finland;* [2]*Finnish Safety and Chemicals Agency, Helsinki, Finland and* [3]*University of Turku, Turku, Finland.*

The fungi *Fusarium* spp. are plant pathogens which may synthesize mycotoxins with various toxic effects. In Scandinavia, Moniliformin (Mon) is a common contaminant in cereals, although at low levels. MON is structurally related to pyruvate and inhibits the incorporation of pyruvate into the TCA cycle, hereby altering the energy metabolism of cells. To assess possible health risks of Mon, an acute and a subacute oral toxicity study of MON were conducted, adapting OECD guidelines 423 and 407. The acute toxicity of MON was studied using Sprague-Dawley rats (3 male animals/group), dosed with synthetic MON by gavage. The starting dose was 50 mg/kg b.w., followed by a lower dose (5 mg/kg b.w). Our findings indicate that Mon is acutely toxic to rats and falls into GHS-category 2 with a LD50 cut-off -value of 5-25 mg/kg bw. The high dose caused muscular weakness, respiratory distress, cardiovascular changes and sudden death due to arrhythmia. Animals receiving the low dose did not show any clinical signs. The clearance of MON into urine was fast as 42% of the administered toxin was excreted during the first 6 h post exposure. In the subacute oral study (synthetic Mon, 5 male Sprague Dawley rats, 0, 3, 6, 9, 12, and 15 mg/kg b.w., two satellite groups 12, 15 mg/kg bw, by gavage for 28 days) was used to assess delayed clinical signs. A complete necropsy was performed to all animals and phagocytic activity of neutrophils was assessed as an impact marker of the innate immunity. Doses lower than 9 mg/kg bw did not induce clinical signs, however, the phagocytic activity of neutrophils was reduced in all doses (3-15 mg/kg bw). Two of the five rats in the highest dose group died of acute heart failure. Clearance corresponds to the acute study. Preliminary results on 8-OHdG in urine did not indicate a difference between the exposed and control groups. This study shows that repeated low doses of Mon do not induce apparent clinical signs in experimental animals. However, they MON has an adverse effect on the innate immunity, due to the loss of phagocytic activity of neutrophils.

3009 Carrageenan Does Not Increase Reactive Oxygen Species (ROS) or Toll-Like Receptor 4 (TLR4) Signaling in Human Intestinal Cell Lines

J. M. McKim, H. M. Baas, G. P. Rice and J. Pregenzer. *IONTOX, Kalamazoo, MI.*

The food additive carrageenan (CGN) is a high molecular weight (200,000 to 800,000 Da) sulfated polysaccharide consisting of repeating galactose and 3,6 anhydrogalactose units linked by alternating α-1,3 and β-1,4 glycosidic linkages. CGN is considered to be safe in animals and humans. Recently, several *in vitro* studies using human intestinal cell lines, including HT-29 and HCT-8, have reported that CGN increases ROS, binds to TLR4 and induces inflammation. These findings are inconsistent with animal safety studies and are intriguing because CGN is a large molecule that binds tightly to protein, rendering it unavailable for cell interaction. In addition, human intestinal cell lines have extremely low expression of TLR4 and low sensitivity to lipopolysaccharide (LPS), a primary TLR4 agonist. Therefore, the aim of this study was to investigate whether well characterized (high purity and endotoxin free) CGN can induce oxidative stress and activate TLR4 signaling pathways in HT-29 and HCT-8 human intestinal cell lines. Cells were grown under recommended culture conditions with 10% serum. Cells were seeded into 96-well culture plates at 15,000 cells/well and cultured until they were confluent. CGN (lambda, kappa, and iota) was tested at 0.1, 1, and 10 µg/mL. LPS (500 and 1000 ng/mL) was used as a positive control and all exposures were for 24 hr. Cellular ATP was used to assess cell viability. Chloromethyl 2′, 7′-dichlorodihydrofluorescein diacetate (CM-DCFDA) was used to measure ROS. Changes in IL-8, TNF-α, IL-6, and CCL2 mRNA were measured by qRT-PCR. In HT-29 cells, exposure to CGN did not induce any of the gene markers. In HCT-8 cells, a similar gene response was observed with no statistically significant changes detected. LPS induced IL-8 by 2-4 fold and this could be inhibited by the TLR4 inhibitor TAK242. At 0.1 and 1.0 µg/mL CGN reduced oxidative stress while at the 10 µg/mL concentration production of ROS was equivalent to control values. No cytotoxicity was observed for any of the test agents. In conclusion, CGN does not activate TLR4 signaling or cause oxidative stress in HT-29 or HCT-8 cell lines and these findings are consistent with published animal safety studies.

3010 Linking a Toxic Glucose Metabolite to Glycemic Responses in an Omnivore Compared to a Carnivore

J. M. Briens[2], K. Desai[2] and L. Weber[2]. [1]*College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada and* [2]*Toxicology, University of Saskatchewan, Saskatoon, SK, Canada.*

Carbohydrates are a key ingredient of pet feed making up between 30-60% of dry pet foods. Cats and dogs are often fed similar diets despite the fact that dogs are omnivores and cats are carnivores. Starches are a main component of carbohydrates, and are the predominant nutrient responsible for increasing post prandial blood glucose. Pulse starches, such as peas and lentils, are digested more slowly compared to high glycemic starches such as corn and rice, which may translate into lower post-prandial blood glucose in cats and dogs. When blood glucose levels increase some may be shunted into producing Methylglyoxal, a toxic glucose metabolite. This metabolite is linked to insulin resistance and diabetes in humans. We hypothesize that pulse starches will produce lower glycemic peaks, lower glycemic indices, and decreased levels of Methylgloxal compared to more commonly used pet food starch sources such as corn, rice or wheat. Normal weight mixed breed domestic cats (n = 9), and beagle dogs (n=8) were fasted overnight, then postprandial glycemic and insulinemic responses to 1g/kg BW available carbohydrate from 8 complex carbohydrate sources (lentil, pea, faba bean, corn, rice,

wheat, potato, tapioca) were tested and compared to a simple carbohydrate control (glucose). Peak blood glucose levels were significantly higher for both glucose and rice compared to all other starches in both cats and dogs. Blood glucose time to peak level was significantly longer for lentil and wheat than other starch sources in cats. Blood glucose time to peak was significantly shorter in glucose, rice, corn and wheat compared to pulse starches in dogs. Methylglyoxal levels increased 60 min postprandial in both cats and dogs following a glucose load. This data from our lab is the first to report postprandial methylglyoxal levels in cats and dogs. The major findings of this study are that pulse starches help to control postprandial blood glucose levels compared to common starch sources in pet foods such as rice and wheat, thereby potentially promoting satiety, better weight control and health benefits in cats and dogs.

PS 3011 Toxicologic Evaluation of a Nature-Identical L-(+) Ergothioneine: Mironova EGT+™

M. R. Bauter[4], P. A. Marone[3], S. M. Weisman[1], D. Merrill[4], L. Koetzner[4], J. Trampota[2] and O. Mendes[4]. *[1]Innovative Science Solutions, Morristown, NJ; [2]Mironova Labs Inc., Fairfield, NJ; [3]Pharmacology and Toxicology, Toxicology and Pathology Associates, Virginia Commonwealth University, Richmond, VA and [4]Toxicology, Product Safety Labs, Dayton, NY.*

L-(+) Ergothioneine is a naturally occurring thiol amino acid with antioxidant properties and potential benefits as a dietary supplement. MironovaEGT+™, as a nature-identical biomimetic of L-(+) ergothioneiene, has been investigated for its ability to induce gene mutations according to a plate incorporation and pre-incubation assay, with *Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherischia coli* WP2uvrA strain, at dose concentrations of 1.58, 5, 15.8, 50, 158, 500, 1580, and 5000 ug/plate with and without metabolic activation. No cytotoxicity or precipitation was observed and no biologically relevant increases in revertant colony numbers of the test article were noted in any of the five tester strains used, up to the highest dose group. Therefore, MironovaEGT+™ did not cause gene mutations by base pair changes or frameshifts in the genome of the strains used, and was considered to be non-mutagenic in the reverse bacterial mutation assay. In a repeat-dose, 90-Day gavage study at dose levels of 0, 400, 800, and 1600 mg/kg/day in Sprague-Dawley rats, in which dose-proportional systemic absorption was confirmed by plasma analysis, there were no adverse clinical, body weight/gain, food consumption and efficiency, clinical pathology, or histopathological changes, associated with the administraiton of MironovaEGT+™. Therefore, the NOAEL for MironovaEGT+™ adminsitered over 90 days was 1600 mg/kg/day, the highest dose tested for male and female rats.

PS 3012 Effects of Novasil Plus and Aflatoxin Exposure on Dry Matter Intake, Milk Production and Milk Composition

C. R. Maki[4], A. D. Thomas[2], S. E. Elmore[4], A. A. Romoser[4], R. B. Harvey[3,4], H. A. Ramirez-Ramirez[2,4] and T. D. Phillips[4]. *[1]AgiLife Research, Texas A&M, College Station, TX; [2]Animal Science and Veterinary Technology, Tarleton State University, Stephenville, TX; [3]Food and Feed Safety Research, US Department of Agriculture, College Station, TX and [4]Veterinary Medicine and Biomedical Sciences, Texas A&M, College Station, TX.*

Fifteen primiparous crossbred dairy cows that were 114 ± 14 d in milk and weighed 662 ± 52 kg were used in a replicated 5×5 Latin square to test the efficacy of NovaSil Plus (NSP) for the reduction of aflatoxin M1 (AFM1) in milk and the effect of NSP on milk composition. The experiment consisted of five 14 d periods: d 1-7 were considered for data collection, and d 8-14 were considered a wash-out phase. In each period, cows were randomly assigned to 1 of 5 dietary treatments: 1) control (CON), composed of a basal total mix ration (TMR); 2) NSP diet (NSP-1%), composed of TMR plus 230 g of NSP; 3) aflatoxin diet (AFD), composed of the TMR plus AF challenge; 4) low dose NSP with AF (NSP-0.5%+AFD), composed of TMR plus 115 g of NSP and AF challenge; 5) high dose NSP with AF (NSP-1%+AFD), composed of TMR plus 230 g of NSP and AF challenge. Milk yield and dry matter intake were similar across treatments averaging 21.1 ± 1.33 kg/d and 19.7 ± 0.56 kg/d, respectively. Concentration of milk fat, protein, and lactose were similar across treatments with averages of 4.91 ± 0.20%, 3.85 ± 0.10%, and 4.70 ± 0.06%, respectively. Concentration of vitamin A averaged 0.28 ± 0.03, mg/L and riboflavin concentration averaged 1.57 ± 0.13 mg/L across treatments. The concentration of minerals in milk were similar for all treatments. Cows fed CON and NSP-1% yielded the lowest concentration of AFM1 in milk with 0.03 and 0.01 ± 0.06 µg/L. Addition of NSP reduced milk AFM1 from 1.10 ± 0.06 µg/L with the AF diet to 0.58 and 0.32 ± 0.06 µg/L with the NSP-0.5%+AF and NSP-1%+AF diets, respectively. Excretion of AFM1 was reduced by NSP; mean values were 24.38, 11.86, 7.38, 0.64, and 0.23, ± 1.71 µg/d, for AFD, NSP-0.5%+AFD, NSP-1%+AFD, NSP-1%, and CON, respectively. More specifically, 1.07 ± 0.08% of the daily AF intake was transferred to the milk of cows consuming the AFD, whereas the AF transfer rates in milk from cows that consumed the NSP-05%+AFD and NSP-1%+AFD were 0.52 and 0.32 ± 0.08%. Results from this research demonstrate that feeding NSP to lactating cows may be an effective strategy to reduce AFM1 transfer into milk along with aflatoxin exposures in young humans and animals.

PS 3013 Characterization of Adipocyte Proteomic Responses to Fructose and/or Glucose

L.-R. Yu, Y. Gao, G. T. Nolen, Z. Cao and V. Varma. *Division of Systems Biology, National Center for Toxicological Research, US FDA, Jefferson, AR.*

Fructose has deleterious metabolic effects in the liver and has been implicated in obesity. Adipose tissue, which plays a key role in the metabolic regulation of obesity, is one of the extra-hepatic target organs that can take up and metabolize fructose. However, the specific molecular alterations occurring in adipocytes in response to fructose treatment and the differential effects of fructose compared to that of glucose are yet to be fully elucidated. In this study, we characterized the adipocyte proteomic changes induced by fructose or glucose treatment. Human Simpson-Golabi-Behmel syndrome (SGBS) preadipocytes were differentiated to adipocytes in the absence (control) or presence of 5, 10 mM fructose or glucose added to the differentiation medium containing 5 mM glucose which represents the normal blood glucose concentration. Proteins from the control and treated adipocyte groups were analyzed by stable isotope labeling and 2D LC-tandem mass spectrometry. Approximately 1200 proteins were quantified from the control or each treatment groups with the false discovery rate (FDR) threshold $q<0.01$. With a significant protein abundance change of 1.4 fold, metabolic network analysis revealed that specific metabolic networks significantly affected were correlated with the type of hexose while a few networks were commonly affected. In general, both 5 and 10mM fructose treatments were more significantly associated with lipid and triacylglycerol metabolism. While both 10 mM fructose and 10 mM glucose treatment were associated with carbohydrate and branched-chain amino acids metabolic pathways, glucose treatment showed more significant correlation to tricarboxylic acids (TCAs), glycolysis and gluconeogenesis, and pyruvate metabolism. Furthermore, a set of 12 proteins were also identified to have differential expression between additional fructose and additional glucose treatment at both concentrations (5 and 10 mM). In conclusion, these quantitative proteomics data provide insights to both differential as well as common cellular proteomic responses to fructose and/or glucose treatment in adipocytes.

PS 3014 Rice Bran Fermentation Enhances *Lactobacillus*-Mediated Reduction of *Salmonella typhimurium* Growth *In Vitro*

N. J. Nealon, E. P. Ryan and C. Worcester. *Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

Salmonella typhimurium is a one health concern, and multidrug resistance necessitates sustainable ways to combat infections. One involves the probiotic *Lactobacilli* that promotes gut health by secreting bioactive molecules into its environment. *In vitro* studies demonstrate that *Lactobacilli* produces metabolites that reduce *Salmonella* growth, and prebiotics may enhance this effect. Therefore synbiotics, the combination of a probiotic and prebiotics, may serve as natural ways to protect against *Salmonella*. Rice bran is an ideal prebiotic source, because it has known health benefits, is produced globally, and has varietal differences in oligosaccharides. Initially, to evaluate how rice bran influences *Lactobacillus*-secreted molecules, we performed an analysis of fatty acid changes during fermentation. After *L. Rhamnosus* fermentation with Calrose rice bran, C20:3 increased ($p<0.05$). C20:3 commonly exists as the isoform omega-3 eicosatrienoic acid, which has roles in reducing inflammation. Given the potential species differences in production of bioactive lipids, we then investigated how synbiotic composition influenced *Salmonella* growth. *L. Fermentum, L. Paracasei* and *L. Rhamnosus* were added to MRS supplemented with LTH or Calrose rice bran extract. Spent supernatant was collected, filtered, pH titrated to and added at different concentrations to *Salmonella*. All treatments were compared to pH-titrated sterile MRS. *Salmonella* growth was evaluated over 16 hours via optical density and plate counts. Results suggest that probiotic fermentation of rice bran reduces Salmonella growth in a probiotic, rice bran, and dose-dependent manner. With LTH rice bran, an 80% concentration of *L. Paracasei* supernatant demonstrated the largest

amount of reduction (p <0.05), while 80% *L. Fermentum* did in Calrose rice bran (p<0.05). Currently, a non-targeted, global metabolomics approach, using GC-MS, is being conducted to evaluate what molecules in the supernatant are driving these effects. We predict that there will be different levels of the same metabolites, primarily fatty acids, between treatments as well as novel metabolites within each treatment. Collectively, *Lactobacillus* and rice bran synbiotics have the potential to serve as novel, sustainable ways to protect against enteric pathogens.

3015 Iron Supplements and Magnesium Peroxide: An Example of a Hazardous Combination in Self-Medication

M. F. Vrolijk[3], A. Opperhuizen[2], E. H. Jansen[1], A. Bast[3] and G. R. Haenen[3]. [1]*National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands;* [2]*Netherlands Food and Consumer Product Safety Authority (NVWA), Utrecht, Netherlands and* [3]*Department of Pharmacology and Toxicology, Maastricht University, Maastricht, Netherlands.*

Background: The use of self-medication, which includes dietary supplements and over-the-counter (OTC) drugs, is still on the rise, while safety issues are not well addressed yet. This especially holds for combinations in self-medication. For example, iron supplements and magnesium peroxide both produce adverse effects via the formation of reactive oxygen species (ROS). This prompted us to investigate the adverse effects of the combination of three different iron supplements with magnesium peroxide on ROS formation. Methods: Hydroxyl radical formation by the three iron supplements either combined with magnesium peroxide or alone, was determined by performing a deoxyribose assay. Free iron content of iron supplements was determined using FerroZine assay. In order to determine the hydrogen peroxide formation by magnesium peroxide, a ferrous thiocyanate assay was performed. Finally, electron spin resonance spectroscopy (ESR) was performed to confirm the formation of hydroxyl radicals. Results: Magnesium peroxide induces the formation of the ROS hydrogen peroxide. All three iron supplements induced the formation of the extremely reactive hydroxyl radical, although the amount of radicals formed by the different supplements differed. It was shown that the combination of iron supplements with magnesium peroxide gives an increased radical formation. ESR confirmed the formation of hydroxyl radicals when magnesium peroxide and iron are combined. Conclusion: All three iron supplements induced the formation of labile iron, which then induced the formation of hydroxyl radicals. Magnesium peroxide gives the formation of hydrogen peroxide, which increased the amount of hydroxyl radicals formed by the iron supplements. Hence, combining these two compounds may cause increased tissue damage in the intestines. More attention should be given to the combinations in self-medication.

3016 Changes in Gene Expression of Enzymes Involved in the Metabolism of Neurotransmitter in Rat Progeny from Dams Fed Different Levels of Dietary Vitamin B6

M. R. Almeida[1,2], V. P. Venancio[2], C. S. Park[1], C. Crane[1], L. Mabasa[1], M. L. P. Bianchi[2] and L. M. Antunes[2]. [1]*Department of Animal Sciences, North Dakota State University, Fargo, ND and* [2]*Department of Clinical Analyses, Toxicology and Food Science, University of São Paulo, Ribeirao Preto, Brazil.* Sponsor: D. P. Oliveira.

Maternal vitamin B6 deficiency results in teratogenicity and neurochemical imbalance of neurotransmitters in the brain of the rat progeny. Furthermore, this vitamin may influence the availability of choline, which is a precursor of the neurotransmitter acetylcholine. In the present study we aimed at evaluating some toxicological parameters of the maternal vitamin B6 deficiency or supplementation in offspring by assessing survival rate, body weight gain, relative liver weight, plasma homocysteine concentration and expression of gene encoding enzymes involved in neurotransmitter metabolism. Moreover, brain choline and acetylcholine concentrations were assessed. Female Wistar rats were fed three different diets: control (6 mg vitamin B6/kg), supplemented (30 mg vitamin B6/kg) or deficient (0 mg vitamin B6/kg) diets for four weeks before pregnancy through lactation. The male pups were euthanized 10 (newborn pups) or 100 days (adult offspring) after birth. We found a high plasma homocysteine concentration in addition to reduced survival rate and body weight gain in newborn pups from dams fed vitamin B6-deficient diet. There was increased glutamate decarboxylase 1 (Gad1) and decreased tryptophan hydroxylase 1 (Tph1) mRNA levels in hippocampus of newborn pups from dams fed deficient diet. The maternal vitamin B6 deficiency modulated expression of genes involved in the glutamatergic pathway by up-regulating Glul (glutamate-ammonia ligase) and down-regulating Gls (glutaminase) in newborn offspring. Vitamin B6 supplementation or deficiency did not change brain choline and acetylcholine levels. In conclusion, a maternal vitamin B6 deficiency modulates expression of genes enconding key enzymes involved in the GABAergic, glutamatergic and serotonergic pathways. Financial support: FAPESP and CNPq.

3017 Assessing Bioactivities and Exposure Profiles of Fruit and Vegetable Juices

B. A. Wetmore[1], M. J. DeVito[3], M. Xia[2], B. Parks[1], R. Huang[2], K. Houck[4], R. R. Tice[3], R. Judson[4], R. S. Thomas[4] and M. E. Andersen[1]. [1]*Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, NC;* [2]*National Center for Advancing Translational Sciences, NIH, Rockville, MD;* [3]*National Toxicology Program, NIEHS, Research Triangle Park, NC and* [4]*Office of Research and Development, US EPA, Research Triangle Park, NC.*

The Tox21 and ToxCast programs have generated *in vitro* screening data for thousands of chemicals to aid hazard identification and testing prioritization. These data, combined with *in vitro-in vivo* extrapolation (IVIVE) using *in vitro* pharmacokinetic data and reverse dosimetry, can be used to infer possible adverse responses and the external concentrations required to elicit them. Similar evaluations of natural product bioactivities hold value in guiding data interpretation. Juices extracted from 30 organically grown fruits and vegetables were screened in concentration-response across Tox21 and ToxCast cell-based assays to compare juice bioactivities against those of xenobiotics. Across the subset of Tox21 assays screened (Ahr, AR, aromatase, CAR, ER), beets, garlic, broccoli and sweet potatoes elicited the most extensive bioactivities. Across the suite of transcription factors assessed in the Attagene multiplexed transcription reporter system, widespread activity was observed across the PPARg, Nrf2/ARE, AhR, and ERa assays. Similar ERa potencies were observed across the two platforms for the three most potent agents - soybeans, edamame and bean sprouts. To relate *in vitro* concentrations to administered dose, the juice yield per g item was used to approximate % juice present in systemic circulation after eating, assuming complete uptake and no first-pass metabolism. To achieve systemic concentrations, of 1.1%, the ERa AC50 for apples, 1 cup would need to be consumed. Assuming a daily exposure to azoxystrobin (a commercial fungicide) at maximal residue levels across 20 fruits and vegetables (~9 mg/kg produce), 68 kg of apples are needed to give the azoxystrobin oral equivalent (8.1 mg/kg/day; after IVIVE and dosimetric adjustment) associated with its 17 µM ERa AC50. These data provide context for assessing the *in vivo* relevance of *in vitro* concentration-response and bioactivity results from Tox21/ToxCast and similar screening programs. The noted bioactivities do not necessarily lead to adverse effects. This abstract does not necessarily reflect U.S. Federal agency policy.

3018 Metagenomics Analyses of Phylogenetic and Functional Genomic Changes in Rat Gut Microbiota Induced by Green Tea Polyphenols

J. Wang[1], L. Tang[1], T. C. Glenn[1], C.-L. Shen[3] and J.-S. Wang[1]. [1]*Department of Environmental Health Science, University of Georgia, Athens, GA;* [2]*Interdisciplinary Toxicology Program, University of Georgia, Athens, GA and* [3]*School of Medicine, Texas Tech University Health Science Center, Lubbock, TX.*

Natural polyphenols from plants are beneficial for humans against multiple diseases and adverse health effects. Gut microorganisms are crucial to human metabolism and bioavailability of polyphenols. However, roles of gut microbiota on beneficial effects of polyphenols have only recently been suggested. The objective of this study was to assess the phylogenetic and functional genomic modifications induced by long-term treatment with green tea polyphenols (GTP) in a rat model. Female ovariectomized Sprague Dawley rats (3 months old) were randomly allocated into three groups (n=15). Rats were fed either with regular water (as the control), 0.75% or 1.5% GTP (weight/volume) in water for 6 months. Rats were sacrificed at the baseline and 6 months to obtain fecal samples from the mid-gut. Microbial DNA was extracted and 16S rRNA gene sequencing and whole-genome shotgun sequencing were performed. Analysis of biodiversity in gut microbial communities showed that Phylogenetic Diversity and Shannon Index are significantly decreased in GTP treated groups in a dose-dependent manner. Analysis of community phylogenetic relationship using UniFrac index showed clear clustering according to GTP doses. In addition to the decrease of Firmicutes to Bacteroidetes ratios at phylum level in dosing groups, abundance of many microbial species were significantly modified as

detected by MetagenomeSeq. Analysis of functional genomes in microbial communities revealed that microbial genes related to polyphenol bioavailability and multiple beneficial effects were accumulated in GTP-dosing groups. These results suggest that gut microbiome can be modified by GTP to promote beneficial effects.

3019 Nutritional and Phytochemical Compositions of Fish Feed with Varying Levels of *Ocimum gratissimum* Inclusions

A. O. Ajiboye[2], B. O. Otegbayo[2] and O. J. Adeyemo[2]. [1]*Animal Science & Fisheries Management, Bowen University, Iwo, Osun State, Nigeria, Iwo, Nigeria and* [2]*Food Science and Technology, Bowen University, Iwo, Osun State, Nigeria, Iwo, Nigeria.* Sponsor: F. Ajani.

Background: Ocimum gratissimum is a widely tropical plant cultivated and known for its medicinal and livestock uses for both man and animals. This study was designed to investigate the effects of O. gratissimum on proximate composition, microbial properties and phytochemical constituents of the fish feeds. Methods: The proximate compositions (crude protein, crude fibre, ether extract, ash and moisture contents), and phytochemical constituents (tannins, saponins, flavonoids, terpenoids and alkaloids) of the feeds were determined according to standard procedures. Fish feeds were compounded with varying levels of O. gratissimum inclusions (0%, 5%, 10%, 15%, 20%) and were denoted T1, T2, T3, T4 and T5 respectively. Data collected were analyzed using one-way analysis of variance (ANOVA) and Duncan multiple range test was used to separate the differences among the means at 5%. Results: The values of phytochemicals in fish feeds showed that tannins (0.77g) and alkaloids (25.75g) were significantly higher $p<0.05$ in T5 than other treatments. Saponins values were significantly higher $p<0.05$ in T2 (0.87g) and T4 (1.60g) than other treatments. The values for flavonoids and terpenoids were not significantly different among the treatments. The crude protein (49.37%) and crude fat (26.37%) in the fish feed were significantly higher $p<0.05$ in T5 than other treatments. The values for crude fibre and ash in the fish feed were significantly higher $p<0.05$ in T4 (5.70%), (4.90%) and T5 (8.77%), (6.97%) respectively. The moisture content was significantly higher $p<0.05$ in T2 (8.83%) and T5 (10.60%) than other treatments. Conclusions: The findings of this study suggest that T5 achieved the best inclusion level of O. gratissimum for proximate compositions and phytochemical constituents of the fish feeds.

3020 Effects of Clays Potentially Used as Food Contact Materials on the mRNA Expression of Selected Genes and Micronucleus Induction in Human Hepatoma Cells

S. Maisanaba[1], M. Jordá-Beneyto[2], A. M. Cameán[1] and A. Jos[1]. [1]*University of Sevilla, Sevilla, Spain and* [2]*Area of Packaging Materials and Systems, ITENE, Valencia, Spain.* Sponsor: G. Font.

Packaging in the food industry is experiencing a fast development in order to find improved materials that allow to increase the shelf-life of food commodities. One of the approaches to get this aim is the incorporation of quaternary ammonium salts-modified clays based on montmorillonite (Mt) into the polymers. This improves, among others, the barrier properties. However, food contact materials are subject to strict regulations and a toxicity evaluation with special focus on genotoxicity aspects is required previously to their authorization. Thus, the purpose of this work was to evaluate two organomodified clays (Cloisite®30B and Clay1) ability to induce genomic instability through the cytokinesis block micronucleus cytome assay (CBMN), and their influence in the modulation of several genes involved in genotoxicity and cell death mechanisms in human hepatoma HepG2 cell line after 4 and 24 hours of exposure using the real time quantitative PCR (qPCR). The non-modified clay (Mt) was also evaluated for comparative purposes. Overall, no genotoxicity response or changes in gene expression were obtained for the organomodified clays, whereas Mt (commercially known as Cloisite®Na+) increased the number of micronuclei and deregulated different genes. Further studies are highly needed to elucidate and increase the knowledge about the molecular mechanisms of clays toxicity. Acknowledgment: The authors wish to thank Junta de Andalucía (AGR5969) for the financial support. Sara Maisanaba also gratefully acknowledges Junta de Andalucía for her predoctoral grant associated to the AGR5969 project.

3021 Evaluation of Reproductive Parameters in Dairy Cattle Fed Diets Naturally Contaminated with *Fusarium Spp*. and *Zearalenona*

T. Quezada-Tristan[2], H. Rivera-Castillo[2], R. Ortiz-Martinez[2], A. Valdivia-Flores[2], L. Medina-Esparza[1] and C. Cruz-Vazquez[1]. [1]*Instituto Tecnologico El Llano, Aguascalientes, Mexico and* [2]*Clinica Veterinaria, Universidad Autonoma de Aguascalientes, Aguascalientes, Mexico.* Sponsor: G. Pallas-Guzman.

The aim of this study was to evaluate the reproductive performance in Holstein cows fed with a diet naturally contaminated with fungi *Fusarium* mycotoxin: zearalenone (ZEA). In two dairy farms were selected 20 cows in each; the cows were in their second pregnancy. The dairy farm is in The Central Plateau of Mexico. In farm A, the group consumed a diet added with a mycotoxin binder; group B did not. Samples of corn silage, concentrated feed and total mixed ration (TMR) were taken every month during 12 months. ZEA concentration was assessed via competitive ELISA; isolation of fungal colonies was performed and reproductive performance of dairy cows was evaluated. Mean concentrations of ZEA were: corn silage 585 µg/kg and 448 µg/kg; TMR 218 µg/kg y 285 µg/kg and concentrated feed 86.4 µg/kg y 98.2 µg/kg in dairy farm A and B respectively. The frequency of feeds contamination by fungi was 44%, where *Fusarium* fungi represented 9.9%. Average days open was 108 ± 51.9 for the group in farm A while in farm B group was 127 ± 73.3. The average number of services per conception in dairy farm A was 2.1 ± 1.2, whereas in the farm B was 2.6 ± 1.6. The ZEA levels in feed did not show significant effects in reproductive parameters of cows in any dairy farm. These results shows that in the area, there is an important fungal contamination in feedstuffs and it could be cause important mycotoxicosis in dairy cattle. Keywords: *Fusarium*, Zearalenone, Reproductive Parameters, Dairy Cattle.

3022 Combined *In Vivo* Genotoxicity Study of Carvacrol, an Oregano Essential Oil Compound, by the Alkaline Comet and Micronucleus Assays

M. Llana-Ruiz-Cabello, M. Puerto, A. I. Prieto, S. Pichardo, A. Jos and A. M. Cameán. *University of Sevilla, Sevilla, Spain.* Sponsor: G. Font.

The food industry is trying to find natural active substances to develop new active packaging systems to respond to consumer demands. In this concern, oregano essential oil has been selected for this purpose due to its antioxidant and antimicrobial activities. Carvacrol is the main component of this essential oil; therefore its safety should be confirmed by different toxicity assays. In this sense, according to the recommendation from the European Food Safety Authority (EFSA) for the genotoxic assessment of substances in food and feed, the *in vivo* approach is needed to confirm the *in vitro* results. In the present study, the *in vivo* genotoxicity of carvacrol was evaluated in the liver and stomach by the comet assay and on bone marrow of wistar rats for micronucleus assay (MN). Animals of both sexes were exposed to carvacrol (81, 270 and 810 mg/kg BW) orally by gavage, according to recommendations of OCDE 474 and 489. The comet assay provides information about mechanisms of genotoxicity since it can detect DNA breaks as well as oxidative DNA damage when enzyme-comet assay are used (Endonuclease III and Formamido pyrimidine glycosylase). The results for the standard comet assay revealed no significant increase in DNA strand breakage at any concentrations assayed. Similarly, post-treatment with End III- or FPG showed no difference between treated and untreated groups. Preliminary results also evidenced absence of genotoxicity in the MN test. Therefore, carvacrol (at low concentrations) could be considered as a good natural alternative to synthetic preservatives used in the food packaging industry. Acknowledgement: the authors wish to thank the Spanish Ministry of Science and Innovation (AGL2012-38357-C02-01) co-financed by FEDER Funds, and Junta de Andalucía (AGR-7252) for the financial support for this study. María Llana Ruiz-Cabello also gratefully acknowledges Junta de Andalucía for her predoctoral grant associated to the AGR7252 project.

3023 Safety Assessment of Proallium AP®: 90-Days Oral Subchronic Toxicity Study in Sprague-Dawley Rats

P. Mellado-García, M. Puerto, S. Pichardo, M. Llana-Ruiz-Cabello, A. Jos and A. M. Cameán. *University of Sevilla, Sevilla, Spain*. Sponsor: G. Font.

Essential oils and their main compounds have been extensively used due to their application in the food industry for improving the shelf life of perishable products. Proallium AP® is a commercial product based in organosulfur compounds which have antimicrobial and antioxidants activities based on vegetal extracts from *Allium spp*. The aim of this study was to investigate the 90-day oral subchronic toxicity effect of Proallium AP®, in 7-week-old Sprague-Dawley rats. The study was conducted in accordance with the guideline 408 of the Organisation for Economic Cooperation and Development (OECD). The rats were divided into four groups (10 rats/sex/group) and Proallium AP®, was administered on diet at doses of 0, 25, 100 and 400 mg/kg bw mixed with neutral gelatine per day. Rats were fed by water and food ad libitum, placed at individual cages and after 90 days, animals were euthanized. There was no mortality, adverse effects on general conditions or changes in body weight, food consumption, water consumption and feed conversion efficiency when the experimental groups were compared to the vehicle control group and no differences in haematology parameters either in white blood cells count when compared with corresponding control values. Moreover, only significant changes ($p<0.05$) were reported in the clinical biochemistry parameters as glucose level (100 mg/kg bw), BUN level at (400 mg/kg bw) in males, as well as in triglyceride level at (400 mg/kg bw) in females. But, these changes were considered not toxicologically significant since they were not consistente. Indicators of hepatic function and renal function were also unchanged. Finally, these results suggest that Proallium AP®, appears to be safe and could be incorporated in active packaging . Acknowledgment: the Spanish Ministry of Science and Innovation (AGL2012-38357-C02-01) co-financed by FEDER Funds and Junta de Andalucía (AGR-7252); and the Microscopy Service of CITIUS from the University of Seville for technical support.

3024 Inactivation of Pathogenic Bacteria on Media Containing Fruit Juices and Spices Using Radiant Catalytic Ionization

B. L. Benjamin[1], A. Babel[1], R. Hardval[1], L. Mummert[1], I. Kuberry[1], L. Schwanke[1], K. Patel[1], R. Patel[1], O. Afolabi[1], D. E. Fulford[1], C. W. Steele[1], C. H. Sommers[2] and W. J. Mackay[1]. [1]*Biology, Edinboro University of Pennsylvania, Edinboro, PA and* [2]*Food Safety Intervention Technologies Research Unit, Eastern Regional Research Center USDA, Wyndmoor, PA.*

Food-borne illnesses are exceedingly prevalent and are an unremitting issue. The World Health Organization (WHO) estimates that 2 billion people worldwide are affected by food-borne illnesses yearly. In order to combat this problem, new sanitizing techniques have emerged in recent years and are being extensively used in a multitude of places to decontaminate contact surfaces. By generating activated oxygen species such as hydrogen peroxide, superoxide anion, hydroxyl radical, and ozone, Radiant Catalytic Ionization (RCI) has come to the forefront of potential solutions for this issue with its countless applications for reducing the number of bacteria. Previous research has shown that RCI causes a 90% killing of food-borne pathogens during a 20 minute exposure. The focus of this study is the examination of the effects of RCI on *Escherichia coli* and *Listeria innocua* grown on different types of media, to simulate common food products. The media that were used for this study include Tryptic Soy Agar (TSA) and TSA infused with organic apple juice, organic grape juice, organic ground turmeric, or organic ground cinnamon. The results of both the apple juice and the grape juice plates indicated a 99.9% killing of both *E. coli* and *L. innocua* in 3 hours. The spices turmeric and cinnamon, both of which are natural antimicrobial agents (African Journal of Biochemistry Research, 4, 172-173), were chosen to explore the possibilities of a synergistic effect between the spices and the food product in the presence of RCI.

3025 Twelve-Week Safety Assessment Study in Dogs with Proprionibacterium Dry Cultured Premix

B. C. Sayers[3], S. Beverly[1], A. Lim[1] and J. Roper[3]. [1]*Dupont Nutrition and Health, St Louis, MO;* [2]*Consulting and Predictive Sciences, Dupont Haskell Global Centers for Health and Environmental Sciences, Newark, DE and* [3]*Human Health and Nutrition Sciences, Dupont Haskell Global Centers for Health and Environmental Sciences, Newark, DE.*

Proprionibacterium are gram-positive non-spore forming bacteria. A subgroup of this genus includes *P. freudenreichii, acidipropionici, jensenii* and thoenii, and is commercially used to produce fermented foods including cheese. *P. acidipropionici* has been well studied as a commensal organism in the gastrointestinal tract of mammals including cattle and humans and has been characterized as playing a positive role in lipid metabolism, immune response and the ability to reduce nitrate. These benefits, as well as an established history of safe use as a traditional starter culture, have identified *P. acidipropionici* as a useful probiotic strain candidate. Dry cultured premixes are an example of probiotic food additives. Dry cultured premixes comprise spray-dried whole-fraction fermentation products of *Propionibacterium acidipropionici* alone or in combination with *Lactobacillus acidophilus*. This safety study was designed to support the use of *P. acidipropionici* premix as a pet food additive. Naïve beagle dogs (n = 4/sex/group) were administered dry cultured premix via capsule at 0, 64, 320 or 640 mg/kg/day for 12 weeks with a four-week recovery period. All animals survived to study termination. No test substance related clinical observations, differences in food consumption and food efficiency, or ophthalmoscopic abnormalities were observed. During dosing, male dogs administered 640 mg/kg/day weighed less and gained less weight than control males. No effects on body weight or body weight gain were observed in males administered 64 or 320 mg/kg/day or in females in any treatment group. No test substance effects were observed in hematology, coagulation, clinical chemistry or urinalysis parameters. The body weight/body weight gain effects observed in males administered 640 mg/kg/day were not considered adverse. As such, under the conditions of this study, the no observed adverse effect level (NOAEL) of *P. acidipropionici* dry cultured premix administered orally was 640 mg/kg/day.

3026 The Effects of Residual Amounts of Tetracycline on the Permeability of Intestinal Epithelial Cells and Gene Expression Regulating Cell Barrier Function

C. Thomas[2], K. Gokulan[2], K. Williams[2], C. Cerniglia[2], S. Khare[2] and S. Pineiro[1]. [1]*College of Veterinary Medicine/US FDA, Rockville, MD and* [2]*National Center of Toxicological Research, Jefferson, AR.* Sponsor: M. Manjanatha.

The use of veterinary antimicrobial drugs in food-producing animals may result in antimicrobial drug residues (ADR) in or on the edible tissues derived from treated animals (meat, milk, eggs). There is an ongoing concern to determine its contribution to the emergence of antimicrobial-resistance in pathogenic bacteria, commensal bacteria, and cause adverse effects on the gastrointestinal tract of human consumers. This study was initiated to assess the effects of varying concentrations (two doses below and above the residual level (1.5 µg/mL)) of tetracycline on the integrity of the epithelial barrier. The hypothesis of the current project is that varying concentrations of tetracycline can directly influence the integrity of the epithelial cell barrier by manipulating genes that are involved with the homeostasis of the intestine. Human T-84 epithelial cells were used in order to study barrier function of the intestinal epithelium. We took advantage of the transwell system which replicates the barrier between the lumen of the intestine and the ileal mucosa, which is a hub for the circulatory system. This system, through measuring trans-epithelial resistance (TER) in polarized cells, replicates the epithelial cells lining the gastrointestinal tract. These polarized cells were exposed to varying concentrations (0.015, 0.15, 1.5, 15, 150 µg/mL) of tetracycline and TER was measured at several time points (0, 1, 2, 3, 6, 24, 48 hrs). Furthermore, RNA was extracted to analyze the expression of genes involved in the maintenance of cell- cell junctions. The results showed concentration and time dependent changes in the permeability of the intestinal epithelial cells. Gene expression results showed the differential regulation of the genes involved in the maintenance of Cell-Cell junctions. Our research highlights the importance of evaluating toxicity of residual concentrations of antimicrobial agents in food that are potentially exposed to consumers, and provide data as guidance for human food safety assessments.

3027 Bioavailability of Methylmercury From Commonly Consumed Seafood: an *In Vitro* Investigation of Gastrointestinal and Colonic Digestion

M. A. Bradley, M. Siedlikowski and N. Basu. *School of Dietetics and Human Nutrition, McGill University, Montreal, QC, Canada.*

Seafood provides essential nutrients, but is also contaminated with methylmercury (MeHg). Current risk assessments for ingested MeHg assumes it is 100% bioavailable in the human gastrointestinal tract. However, recent studies on MeHg bioavailability suggest otherwise. These studies were mostly performed outside of North America, on seafood not commonly consumed in North America, and focus almost exclusively on bioaccessibility (digestive processes) rather than bioavailability (cumulative digestive + absorptive processes). We aimed to evaluate both MeHg bioaccessibility and bioavailability from edible tissue of the top 10 most consumed types of seafood in the United States, identified by a previous survey. We used an *in vitro* model of human digestion including gastric and small intestinal phases; some digestions also included a colonic phase. Bioaccessible MeHg was measured in the soluble fraction resulting from these digestions. Soluble fraction was added to a Caco-2 Transwell assay to assess MeHg bioavailability. After gastrointestinal digestion, mean MeHg concentrations ranged from 0.73 ng/g (scallop) to 527 ng/g (canned white tuna) in undigested seafood, from 0.73 ng/g (scallop) to 366 ng/g (fresh tuna) in bioaccessible fraction, and from 0.34 ng/g (scallop) to 266 ng/g (fresh tuna) in bioavailable fraction. MeHg bioaccessibility ranged from 50% (canned white tuna) to 100% (shrimp, scallop), and bioavailability from 29% (crab) to 67% (salmon). In preliminary studies, colonic digestion of fresh tuna reduced MeHg bioaccessibility by 12% relative to gastrointestinal digestion only. In conclusion, both digestive and absorptive processes seem to have a role in reduced MeHg bioavailability, thus suggesting that ingested MeHg is less than 100% bioavailable. More accurate estimates of MeHg bioavailability can help properly balance the toxicological risks and the nutritional benefits of consuming seafood, though additional research is needed before use in risk assessment.

3028 28-Day Repeated Dose Acute Toxicity of the Food Processing-Induced Chemical 2-Monochloro-1,3-Propanediol (2-MCPD) in F344 Rats

J. Raju, J. Roberts, M. Taylor, D. Caldwell, D. Patry, E. Mutzke, J. Eastwood, R. Bronson, G. Cooke and R. Mehta. *Food Directorate, Health Canada, Ottawa, ON, Canada.*

MCPDs are known food contaminants resulting from heat-induced processes and generated by a Maillard reaction; high levels have been reported in vegetable oils. Toxicity data for 3-MCPD are available, but a detailed toxicological evaluation of 2-MCPD is lacking. A 28-day subacute rodent study was undertaken to assess potential health hazards of dietary 2-MCPD exposure. Male and female Fischer F344 rats were fed AIN-93G diet containing no MCPD (controls), or 2-MCPD (25-200 mg/kg BW/day) or 3-MCPD (25 and 200 mg/kg BW/day). Within the first week of exposure, female rats at 100 and 200 mg/kg 2-MCPD became moribund and were euthanized; male rats at these high doses were then also excluded from the study. At necropsy, blood and tissues were collected for analysis. Kidney toxicity was observed in both sexes with 2-MCPD (25 and 50 mg/kg) as corroborated by higher kidney weights and marked changes in serum creatine kinase, amylase, and blood urea nitrogen. Serum ALT, AST, HDL, cholesterol and triglyceride levels were also differentially modulated by 2-MCPD alluding to hepatic and systemic toxicity. Hematological changes included significantly lower red blood cells, hemoglobin and hematocrit values in males, and platelet and mean platelet volumes in females at 50 mg/kg 2-MCPD; and a significantly higher B-cell and lower natural killer cell count in females at 50 mg/kg 2-MCPD. A few non-neoplastic lesions with minimal to moderate scores were observed in the kidney (50 mg/kg in males), heart (50 mg/kg in females) and thyroid (25 and 50 mg/kg in males and 50 mg/kg in females). Such pathology was not observed in 3-MCPD rats at 25 mg/kg BW/day. Our results show kidney toxicity and sex- and 2-MCPD-specific changes at the lowest dose of 25 mg/kg BW/day for a number of outcomes tested. These data will assist the hazard characterization of 2-MCPD by regulators and provide an impetus to investigate its role when exposed chronically.

3029 Lung Inflammation and Toxicological Assessment in A/J Mice in Response to Chronic Exposure to Mainstream Aerosol from Candidate Modified Risk Tobacco Product and Smoke from Conventional Cigarettes

E. T. Wong[1], K. Luettich[2], K. Trivedi[2], E. Guedj[2], Y. Xiang[2], A. Elamin[2], E. Veljkovic[1], A. W. Hayes[3], P. Leroy[2], P. Vanscheeuwijck[2], M. Peitsch[2] and J. Hoeng[2]. [1]*Philip Morris International Research Laboratories Pte Ltd, Singapore, Singapore;* [2]*Philip Morris International, Products S.A., Neuchatel, Switzerland and* [3]*Spherix Consulting, Rockville, MD.*

Chronic exposure to cigarette smoke is the leading cause of lung cancer and chronic obstructive pulmonary disease. The A/J mouse model is used to evaluate lung inflammation, lung cancer development, toxicological impact and the underlying molecular changes upon life-time exposure to cigarette smoke (CS) from a reference cigarette 3R4F or to aerosol from the Tobacco Heating System (THS) 2.2, a candidate modified risk tobacco product (cMRTP). A/J mice were exposed for 6 hours per day for 5 days per week with planned total exposure duration of 18 months. Quantification of pulmonary inflammation, lung function tests, histopathological evaluation, toxicological assessment as well as transcriptome and proteome analysis of respiratory tract organs were performed at interim dissections. Exposure to CS induced changes in red blood cell profiles that are consistent with high carbon monoxide in CS smoke but were not observed following THS2.2 aerosol exposure. Exposure to CS also resulted in pulmonary inflammation, altered lung function and molecular changes that are suggestive of emphysema but only minimal effects were observed following THS2.2 aerosol exposure. Significant changes in the airway transcriptome and proteome were observed following CS exposure, but only few genes and proteins were altered following THS2.2 aerosol exposure. Biological network analysis identified perturbed molecular processes that are consistent with CS exposure, but only minor perturbations of cell stress responses were noted following THS2.2 aerosol exposure. In summary, lung inflammation and the associated loss of pulmonary function and molecular perturbations were significantly reduced in mice exposed to aerosol from THS2.2 as compared to CS exposure.

3030 Application of *In Vitro* Cell Stress Assays Using Lung Epithelial Cells for the Assessment of Electronic Cigarettes

M. Taylor, T. Carr, O. Oke, T. Jaunky, F. Lowe and M. D. Gaca. *Pre-Clinical Assessment, British American Tobacco, Southampton, United Kingdom.*

Cigarette smoking is a risk factor for a number of human diseases including heart disease, lung disease and cancer. Although mechanisms underlying these disorders are diverse, each are underpinned by smoke-induced cellular anti-oxidant, pro-inflammatory and apoptotic responses. These responses are mediated by cell signalling pathways, including those controlled by the transcription factors Nrf2 (antioxidant responses) and NF-kβ (inflammatory responses). Using these cell stress endpoints, we are able to compare the relative biological effects of new categories of tobacco and nicotine delivery products with those of traditional cigarettes. In this study we show how we employed these assays to assess a commercially available e-cigarette. Human bronchial epithelial (H292) cells were exposed to positive controls or aqueous extracts (AqE) from a 3R4F reference cigarette or a commercially available e-cigarette (Vype ePen). Oxidative stress was characterised by intracellular glutathione ratio (GSH:GSSG) measurements, intracellular ROS production and transcriptional activation of the Nrf2-mediated Antioxidant Response Elements (AREs) with a luciferase reporter H292 cell line. The inflammatory response was determined by quantification of secreted cytokines IL-1β, IL-6, IL-8 and TNF-α. Apoptotic and necrotic responses were characterised by measuring Caspase 3/7 and live-cell protease activities. All cell stress response endpoints were activated by exposure to positive controls. 34RF reference cigarette extracts produced a concentration-dependent response in all assays. There was a statistically significant difference between almost all treatment groups and the untreated control group in all assays ($P<0.05$).The same cell stress response endpoints were not induced by e-cigarette exposure at any concentration tested, up to 100%. This model was able to distinguish between responses to 3R4F reference cigarette and e-cigarette AqE exposures, demonstrating that this model is suitably sensitive for comparative *in vitro* studies of cigarettes and novel nicotine products.

3031 The Assessment of γH2AX Induction From Conventional and Electronic Cigarette Aerosols

D. Thorne, S. Larard, C. Garcia-Canton, C. Meredith and M. D. Gaca. *Pre-Clinical Assessment, British American Tobacco, Southampton, United Kingdom.*

Exposure systems have been used to assess cigarette smoke aerosols for many years, using a variety of *in vitro* endpoints. These systems produce a more physiologically relevant test matrix compared to traditional methods and as a result, *in vitro* aerosol techniques are widely being developed. Of particular interest are genotoxicity assays such as the γH2AX assay, which may be used to detect DNA damage, a possible precursor to cancer. The γH2AX assay detects DNA double strand breaks (DSB), via phosphorylation of the H2AX histone protein which occurs at the site of the DSB. This study describes the method development and optimisation of the γH2AX assay for assessment of conventional and electronic cigarette (e-cigarette) aerosols. The γH2AX assay was adapted for use using BEAS-2B cells with the Vitrocell® VC 10 air liquid interface (ALI) aerosol exposure system. Exposures were defined in this study as a dosimetric measure rather than routine diluting airflows (L/min). Using quartz crystal microbalance technology, exposures were based on gravimetric dose measurements (µg/cm2) obtained *in situ*. Cytotoxicity was assessed using total cell counts. Conventional 3R4F reference cigarette demonstrated a dose dependent correlation for cytotoxicity and γH2AX induction with increased exposure, at 0, 3, 5, 10, 20 and 40 µg/cm2. Doses 20 and 40 µg/cm2 were excluded due to high cytotoxicity. E-cigarette aerosol generated from a commercially available product (Vype ePen) remained negative for cytotoxicity and γH2AX induction, despite dosing to 150 µg/cm2, 15-fold higher than the equivalent cigarette smoke exposure. All negative controls remained unaffected and the positive control chemical etoposide produced a strong positive increase in γH2AX induction. This study demonstrates a novel approach incorporating dosimetry as an important consideration for *in vitro* product testing. It further demonstrates the successful adaptation to the ALI for the assessment of conventional and e-cigarette aerosols and may prove useful as a rapid high content screening technique for product assessment

3032 Design Features That Reduce Tin and Other Metals in Electronic Cigarette Aerosol

M. Williams[2], A. To[2], K. Bozhilov[2] and P. Talbot[2]. [1]*Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA and* [2]*Central Facility for Advanced Microscopy, University of California, Riverside, Riverside, CA.*

Metals are present in electronic cigarette (EC) fluid and aerosol and may present health risks to users. The purpose of the this study was to measure the amounts of tin, copper, zinc, silver, chromium, and nickel in the aerosol from four brands of EC and to identify the sources of these metals by examining the elemental composition of the atomizer components. Four brands of popular EC were dissected and the cartomizers were examined microscopically. Elemental composition of cartomizer components was determined using integrated energy dispersive X-ray microanalysis, and the concentrations of the tin, copper, zinc, silver, chromium, and nickel in the aerosol were determined for each brand using inductively coupled plasma optical emission spectroscopy. All filaments were made of nickel and chromium. Thick wires were copper coated with either tin or silver. Wires were joined to each other either by tin solder, brazing, or brass clamps. High concentrations of tin were detected in the aerosol when tin solder joints were friable. Tin coating on copper wires also contributed to tin in the aerosol. Tin concentrations in EC aerosols varied both within and between brands. Tin in aerosol was reduced by coating the thick wire with silver rather than tin, placing stable tin solder joints outside the atomizing chamber, joining wires with brass clamps, or brazing rather than soldering wires. These data demonstrate the feasibility of removing tin and other unwanted metals from EC aerosol by altering designs and using materials of suitable quality.

3033 Evaluation of Elements of Toxicological Interest in the Chemical Characterization of Vapor Products

J. M. Wertman, J. Strasser, W. Xie and E. H. Theophilus. *Reynolds American Inc, Winston-Salem, NC.*

Vapor products (e.g., e-cigarettes, tanks) are consumer products of regulatory interest for which there is no consensus on how to conduct a toxicological evaluation. To establish best practices for vapor products evaluation, a literature review was conducted of 1) published chemistry studies focused on elements in vapor products and 2) national and international guidance documents and standards related to control of elemental impurities in other relevant products (e.g., consumer products, pharmaceuticals, medical devices, packaging). The goal of the review was to propose a strategy for evaluation of elements of toxicological interest in vapor products. We propose a 2-prong, data-centric strategy based on 2 main matrices: 1) cartridges/parts in contact with e-liquid/artificial saliva and 2) finished product aerosol. An initial, broad scan of ~45 elements can be conducted to identify any elements above the limit of quantitation (LOQ) in fluids in contact with cartridges/parts using semi-quantitative methods. A follow up or parallel study can be conducted to determine if elements reported > LOQ in the scan are transferred to aerosol. ICP-MS/OES analytical methods are used for these types of studies. When aerosol studies are conducted, an aerosol-generating machine with a 55/30/3 square wave aerosolizing regimen is typically used. While scans can be conducted in research mode, aerosol measurements should use validated quantitative methods. Elements reported > LOQ are risk-assessed (an example of a risk assessment will be presented). A thorough chemical characterization strategy is consistent with existing strategies for other relevant products geared towards control of potential impurities/residuals/contaminants. This strategy strengthens the risk assessment paradigm, helps position tobacco products on the risk continuum, and minimizes the need for animal testing.

3034 Targeted Aerosol Compound Quantitation: An Important Tool in Chemical Characterization of Vapor Products

D. T. Szabo, J. Strasser, W. Xie, J. Wertman, J. Griggs and E. Theophilus. *RJ Reynolds, Winston Salem, NC.*

Vapor products, such as e-cigarettes and tanks, are a rapidly evolving class of consumer products with worldwide regulatory interest. To assess their potential health risks, a stewardship evaluation strategy can involve product and aerosol chemical characterization. The aerosol composition of vapor products is largely predictable based upon a thorough understanding of e-liquids and product design. In addition, a rigorous product stewardship and quality program can eliminate potential contaminants of concern or ensure that unavoidable compounds of concern present negligible health risks to consumers. A series of aerosol chemistry studies were conducted on a variety of vapor product designs and analyzed to guide industry and regulatory assessment of vapor products. Studies used an aerosol-generating machine with a 55/30/3 square wave aerosolizing regimen and GC-MS, LC-MS, and ICP-MS analytical methods. Nearly 100 targeted compounds were screened in the aerosol, representing a wide range of chemical classes, including those present on the FDA Center for Tobacco Products Hazardous and Potentially Hazardous Constituents reportable list for combustible cigarettes. Only a small subset of compounds screened was detected above the limit of quantitation (LOQ). These data support analyzing a shorter list of constituents for product stewardship of vapor products. The inclusion of aerosol constituents beyond those in the current studies may be re-evaluated as the vapor product category evolves. A clear understanding of 1) chemical composition, 2) product design (physical, electrical, electronic, and mechanical) parameters, and 3) manufacturing processes, is required for a rigorous vapor product risk assessment.

3035 Effects on the ApoE-/- Mouse Lung Epigenome of Exposure to a Candidate Modified Risk Tobacco Product, THS 2.2, Compared with Conventional Cigarettes

N. Sierro[3], F. Martin[3], M. Talikka[3], S. Ouadi[3], J. Thomas[3], K. Baumer[3], B. Phillips[2], E. Veljkovic[2], A. W. Hayes[1,3], M. C. Peitsch[3], J. Hoeng[3] and N. V. Ivanov[3]. [1]*Spherix Consulting, Rockville, MD;* [2]*Philip Morris International R&D, Philip Morris International Singapore, Singapore, Singapore and* [3]*Philip Morris International R&D, Philip Morris Products S.A., Neuchatel, Switzerland;* [4]*Scientific Advisory Board, Philip Morris International R&D, Philip Morris Products S.A., Neuchatel, Switzerland.*

Smoking cigarettes is a major risk factor in the development and progression of cardiovascular disease (CVD) and chronic obstructive pulmonary disease (COPD). Candidate modified risk tobacco products (MRTPs) are being developed to reduce smoking-related health risks. The goal of this study was to investigate over an 8-month period the effects of exposure to conventional cigarette smoke (CS) or to the aerosol of a candidate MRTP, Tobacco Heating System (THS) 2.2, on DNA methylation in the lung of apolipoprotein E-deficient mice. In addition to chronic exposure, cessation or switching to THS 2.2 after 2 months of CS exposure were also assessed. High-throughput sequencing of bisulfite treated DNA revealed a gradual increase in the number of hypermethylated CpG loci in DNA extracted from lungs of mice exposed to

CS. The number of hypermethylated CpG loci after 2 month exposure to THS 2.2 aerosol (nicotine concentration matched to CS, 29.9 mg/m3) also increased, however, hypermethylation was limited from 3 month on. Cessation or switching to THS 2.2 both resulted in a decrease in the number of hypermethylated CpG loci. In conclusion, this mouse model indicated that initial exposure to CS or THS 2.2 resulted in hypermethylation of CpG loci in lung, and that continuous exposure to CS further increased the number of hypermethylated CpG loci while continuous exposure to THS 2.2 reverted the CpG methylation level to the level observed in the lung of mice exposed to fresh air. Likewise, cessation or switching to THS 2.2 reverted the hypermethylation of CpG loci.

3036 Xenobiotic Metabolism Responses of Nasal, Oral and Bronchial Organotypic Epithelial Cultures: a Comparison of Tobacco Heating System Aerosol and Combustible Cigarette Smoke

A. R. Iskandar[1], S. Majeed[1], L. Ortega Torres[1], D. Kuehn[1], S. Johne[1], F. Martin[1], Y. Xiang[1], A. Sewer[1], A. Sewer[1], P. Leroy[1], R. Dulize[1], E. Guedj[1], N. V. Ivanov[1], C. Mathis[1], W. A. Hayes[2] , J. Hoeng[1] and M. C. Peitsch[1]. *[1]PMI R&D, Neuchatel, Switzerland and [2]Spherix Consulting, Rockville, MD.*

Metabolism of xenobiotics into reactive intermediates often contributes to toxicity, thus the impact of exposure on the host xenobiotic metabolism machinery is often utilized for toxicity assessment. For the assessment of inhaled agents, organotypic airway epithelial models become relevant as they are cultured at the air-liquid interface and resembling better the airway tissue *in vivo*. Different from conventional cigarette smoking, by which over 6000 compounds are generated from combustion, heating tobacco instead of burning produces lower concentrations of harmful and potentially harmful constituents. In this study, we compare the impact of smoke from 3R4F reference cigarettes to that of the aerosol from a tobacco heating system (THS)2.2, a heat not burn technology-based candidate modified risk tobacco product (MRTP), on the xenobiotic metabolism response. Three human epithelial organotypic tissue cultures (nasal, oral and bronchial) were exposed to cigarette smoke (CS) or THS2.2 aerosol for 28 min. Subsequently, CYP1A1/1B1 activity and gene expression profiles were generated at various post-exposure time points. The results indicate that, at the comparable nicotine concentrations, the activity of CYP1A1/1B1 and gene expression of various CYPs were higher after CS exposure than after exposure to THS2.2 aerosol in all three cultures. Using a xenobiotic metabolism network model and systems biology approach, the transcriptomic data illustrate a greater impact on the xenobiotic metabolism in CS-exposed tissues than THS2.2-exposed tissues. Collectively, our observation in the three *in vitro* cultures suggests that the xenobiotic metabolism responses can discern the potential toxicity profiles associated with CS from that associated with THS2.2 aerosol.

3037 Differential Gene Expression Using RNA-Seq Profiling in a Reconstituted Airway Epithelium, Mucilair, Exposed to Conventional or Electronic Cigarettes Aerosols

A. Banerjee, L. E. Haswell, A. Baxter, A. Parmar, D. Azzopardi, S. Corke, D. Thorne, J. Adamson, M. Gaca and E. Minet. *R&D Centre, British American Tobacco, Southampton, United Kingdom.*

Electronic cigarettes (e-cigs) potentially offer a safer alternative to combustible tobacco. Yet there is limited scientific evidence regarding their relative risk for health. Since epidemiology is retrospective it cannot at this point guide regulatory decisions on novel nicotine delivery products. The advance in Molecular Biology and Computer Sciences offers new perspective to apply innovative approaches to assess adverse biological responses by combining omics screens with knowledge-base biological pathways. Our aim was to compare the effects of cigarette smoke versus e-cig aerosol on gene expression pathways. We therefore measured transcriptomic perturbations in primary airway epithelial cells (MucilAir) after short repeated exposure to cigarette smoke (3R4F) and e-cig (Vype ePen) aerosols matched for puff profile and mass deposition. Deep RNA-sequencing and profiling of secreted inflammatory cytokines was performed for the tests and air controls. For 3R4F and the e-cig, respectively, 123 versus no genes were differentially expressed at FC (fold change)>1.5 and pFDR<0.1 (Benjamini-Hochberg) after adjustment for replicates and recovery time. Since e-cig exposure did not result in any transcriptomic perturbations at pFDR<0.1, the pFDR was increased to 0.5. Even then, only 29 genes were identified for QRT-PCR validation. Gene enrichment analysis was conducted using IPA ingenuity® predicting a significant increase in tumor promotion (z-score>2) for 3R4F. No specific enrichment alert was evident for e-cigs at those exposure conditions. No differential splicing event could be detected. Out of a panel of 33 cytokines screened, 8 were up-regulated (FC>1.5 p-value<0.05) following 3R4F smoke exposure only. In conclusion, e-cigs caused minimum gene expression perturbation compared to a conventional cigarette after short repeated exposure.

3038 Aryl Hydrocarbon Receptor 2 Morpholino Knockdown Reduces the Toxicity of Total Particulate Matter from Cigarette Smoke in Zebrafish (*Danio rerio*) Embryos

A. Massarsky[1], A. J. Bone[1], G. L. Prasad[2] and R. T. Di Giulio[1]. *[1]Duke University, Durham, NC and [2]R. J. Reynolds Tobacco Company, Winston-Salem, NC.*

Cigarette smoking is a major risk factor for many serious diseases and is associated with significant mortality in some smokers; however, there remain knowledge gaps pertaining to the mechanisms leading to its adverse teratogenic effects. The zebrafish embryo is a potentially useful 'bridge model', and has been recently proposed for the study of cigarette smoke toxicity. Our previous study demonstrated that exposure of zebrafish embryos to total particulate matter (TPM) from cigarette smoke leads to deformities, reduced heart rate, increased mortality, and decreased growth. These effects were accompanied by changes to biomarkers of xenobiotic metabolism (cytochrome P4501A (CYP1A) and glutathione-S-transferase activities) and oxidative stress (glutathione concentrations and glutathione reductase activities), suggesting that the aryl hydrocarbon receptor (AHR) pathway and the antioxidant defense system might be important mediators of TPM toxicity. Thus, the first part of this study examines the role of AHR pathway, using the AHR2 morpholino knockdown approach, and the second part investigates the role of oxidative stress, using co-treatments with an antioxidant (N-acetyl cysteine; NAC) or a pro-oxidant (buthionine sulfoximine; BSO). We demonstrate that while the alteration of glutathione concentrations with NAC/BSO does not ameliorate/worsen TPM toxicity, the knockdown of AHR2 reduces TPM teratogenicity. The toxic effects of TPM in nuclear factor erythroid 2-related factor 2 (Nrf2) and CYP1A morpholino-injected embryos are also being investigated. Results to date suggest that the toxicity of TPM is partially mediated by the AHR pathway.

3039 Combined Effects of Cigarette Smoke and SO2 on GM-CSF Release in Human Airway Smooth Muscle Cells

B. A. Rolls[2], L. M. Hallberg[2], M. A. Thompson[1], Y. S. Prakash[1] and B. T. Ameredes[2]. *[1]Mayo Clinic, Rochester, MN and [2]University of Texas Medical Branch, Galveston, TX.*

Rationale: Asthma is a chronic inflammatory airway disease involving dysregulation of human airway smooth muscle cell (HASMC) function, and it is known that asthmatics can be more susceptible to the adverse effects of inhaled pollutants, such as cigarette smoke (CS) and sulfur dioxide (SO2). Prior studies have investigated these toxicants individually; thus, we investigated their combined effects on pro-inflammatory cytokine release in HASMC. Methods: Cultures of primary HASMC from 3 donors at passage 6 or 7 were serum-starved for 24hr, then exposed to CS, SO2 or both combined, re-fed and incubated. CS was administered directly to cultures with air (1/2 cigarette burned for 4 min, total exposure time of 7 min); SO2 was administered at 1 ppm in air (30 min). GM-CSF release was measured 8 and 24 hr after exposures in conditioned media, by ELISA, with data comparisons by t-test. Results: GM-CSF release by HASM averaged ~100 pg/ml with SO2 exposure at 8 and 24 hr, and was not different from that with air, alone. With CS exposure, GM-CSF rose significantly, to levels ranging from 350-1230 and 700-2200 pg/ml, at 8 and 24 hr, respectively. GM-CSF with CS + SO2 at 8 hr was significantly less than CS alone (94-380 pg/ml), suggesting some compensatory mechanism involving SO2. However, this compensatory mechanism was lost with CS + SO2 at 24 hr, when GM-CSF release remained elevated, similar to CS alone (950-2350 pg/ml). Conclusions: We conclude that CS results in a 2-fold increase in GM-CSF release from 8 to 24 hr, but that there is an even greater effect of the combination of CS and SO2 over that same interval, resulting in 6- to 10-fold increments in GM-CSF release in HAMSC, suggesting some interactive effect of CS and SO2 over a period that is relevant to human asthma exacerbations. Supported by: NIEHS T32ES007254, NIEHS 5P30ES006676, The Sealy Center for Environmental Health and Medicine, and the Brown Foundation.

3040 Can *Ex Vivo* Genotoxic Endpoints Complement Respiratory Tract Pathology Following 3 and 6 Week Cigarette Smoke Exposure?

A. Dalrymple[1], P. Ordoñez[3], D. Thorne[1], O. Camacho[1], A. Büttner[2], D. Dillon[1] and C. Meredith[1]. [1]*British American Tobacco R&D Centre, Regents Park Road, Southampton, SO158TL, United Kingdom;* [2]*Histovia GmbH, Schöne Aussicht 5, 51491 Overath, Germany and* [3]*Vivotecnia Research S.L., Parque Científico de Madrid, 28760 Tres Cantos, Madrid, Spain.*

Cigarette smoke (CS) induced DNA damage can be measured in rat lung alveolar type II epithelial cells (AECII) following CS exposure (Dalrymple et.al, 2015). This study was initiated to determine whether DNA damage, Pig-a mutation, *in vivo* micronucleus (MN) or respiratory tract histopathology could be quantified following 3 or 6 week (wk) CS exposure and the feasibility of separate lung lobes for histopathology or Comet assay to reduce animal number. Rats were exposed to sham air (SA) or 0.6mg WTPM/L 3R4F CS for 1h or 2h/day. Following 3 or 6 wk exposure, left lung lobe, nasal cavity, larynx, and trachea were processed for histopathological evaluation. AECII were isolated from right lobes and analysed by the Comet assay. Blood was collected at 6 wk for Pig-a and MN quantification. Histological sections were evaluated blind (semi-quantitative, 5 steps severity grading). DNA damage was statistically significantly increased following 3 or 6 wk CS exposure. Values obtained (mean % tail DNA ± SD) were 3.1%±7.4, 10.1%±14.1 and 10.6%±14.4 for 3 wk and 3.2%±7.3, 13.4%±14.5 and 13.8%±16.2 for 6 wk exposure to SA, 1h CS or 2h CS respectively. The level of CS induced DNA damage was also statistically significantly different when 3 and 6 wk data were compared ($p<0.0001$). Histopathology demonstrated statistically significant exposure effects for the 22 histopathological endpoints evaluated in the nasal cavity, larynx, trachea or lung. Effects observed after 3 wk were, in general, dependent on CS dose (1 vs 2h) and length of exposure (3 vs 6 wk). The level of Pig-a or MN was not significantly increased following CS exposure, p0.566 and p0.078 respectively. In conclusion, DNA damage was the only measured genotoxic endpoint increased by CS. Differences between the 3 and 6 wk time points implies that DNA damage in the rat lung is accumulative. Respiratory tract histopathology was consistent to previous studies. These data suggests that 3 wk combustible CS exposure is sufficient to observe genotoxic responses in AECII and respiratory tract pathology. Reducing exposure time, plus the use of separate lung lobes for DNA damage or histopathology supports a strategy to reduce and refine animals use in product testing.

3041 A Targeted Proteomic Comparison of Human-Induced Sputum from Smokers and Non-Smokers

L. E. Haswell[4], O. M. Camacho[4], W. R. Fields[3], L. Cortes[1], P. Croteau[1], L. McIntosh[1], F. Mohareb[2], D. Chelsky[1], G. Phillips[4] and M. D. Gaça[4]. [1]*Caprion Proteomics, Montréal, QC, Canada;* [2]*Cranfield University, Bedford, United Kingdom;* [3]*R&D, R J Reynolds Tobacco, Winston-Salem, NC and* [4]*R&D Centre, British American Tobacco, Southampton, United Kingdom.*

Background: Smoking is a leading cause of disease worldwide and remains a public health concern. We used a proteomics approach to identify candidate biomarkers of effect in sputum samples from smokers. Differentially expressed proteins may be early indicators of tobacco-related diseases and could be used in future tobacco product assessment assays. Methods: We developed a proteomics assay for sputum samples and performed semi-quantitative shotgun analysis to characterise the protein profile of smokers and non-smokers. Based on results from the identification phase and information from literature, we conducted a multiple reaction monitoring (MRM) assay to measure relative protein abundance. Results: We identified 912 proteins in sputum, of which 714 were detectable in both smokers and non-smokers. Of those proteins represented by three or more peptides, 74 proteins were upregulated and 37 proteins were down regulated in smokers. Quantitative comparisons of the protein content between smokers and non-smokers were performed using the MRM assay. Of the 139 proteins measured, significant differences in protein abundance between smokers and non-smokers were observed for 29 proteins. Differences above 2-fold changes were observed for 18 of those proteins, with four proteins upregulated in smokers above 3-fold change (AL3A1, CATD, K1C16 and CEAM5). Conclusion: 912 proteins identified this study have contributed to the characterisation of the sputum proteome. We observed differences in the protein content between the sputum of smokers and non-smokers, and identified 29 candidate biomarkers that can be further assessed for their suitability as biomarkers of effect to tobacco smoke constituents.

3042 Efficacy of an Inhaled PDE4 Inhibitor, GSK256066, Delivered by a Novel Dry Powder Pre-Clinical Inhalation Delivery System in the Acute Cigarette Smoke Induced Pulmonary Inflammation Model

S. A. Moore[1], S. Jordan[1], D. Rodgers[1], C. Gutteridge[1], R. Armstrong[1], K. Meecham[1], G. Paul[2] and D. Mitchell[1]. [1]*Envigo, Huntingdon, United Kingdom and* [2]*GlaxoSmithKline, Ware, United Kingdom.*

Short term cigarette smoke (CS) exposure in mice has been shown to exhibit similarities to cell and cell-mediated inflammation observed in patients with COPD. Our aim was to evaluate the efficacy of an inhaled PDE4 inhibitor, GSK256066 in the murine 4day CS model. GSK256066 (a PDE4 inhibitor) was delivered as a powder using a novel aerosol generator (CBAG). Female C57BL/6 mice were exposed in a whole body system to a concentration of 3R4F CS (600 µg/L) or air, twice daily (1h per occasion). Lactose or GSK256066 were dosed by nose only inhalation using CBAG twice daily for 30 min, 1 h prior to the start of each CS exposure. Acute CS exposure resulted in a statistically significant ($p<0.001$) neutrophil driven inflammatory response in the airways with elevated Bronchi-alveolar lavage (BAL) levels of KC ($p<0.01$), MCP-1 ($p<0.001$) and IL-6 ($p<0.05$) compared to lactose/air control animals. Exposure to GSK256066 at inhaled doses of 34, 307 and 864µg/kg (all within 15% from target with an acceptable particle size) produced a dose dependent inhibition of pulmonary neutrophilia with maximal efficacy of approximately 50% at 307 and 864µg/kg respectively. BAL KC was reduced by GSK256066 and also by steroid intervention. IL-6 was significantly increased in the test animals compared with vehicle/CS treated animals (peak response at 307µg/kg: 241 vs 17pg/mL). The GSK256066 compound requirement to achieve the high dose was 38mg compared to 875mg for a standard commercial device. In conclusion, the 4day acute CS model induces a robust inflammatory response in the murine lung. GSK256066 significantly attenuated the neutrophil driven airway inflammation. The augmentation of CS induced BAL IL-6 levels remains uncertain. The CBAG offers a significant reduction in test article over other instruments.

3043 Obesity Combined with Cigarette Smoke Exposure Dramatically Alters Cytochrome P450 Activity in the Lung and Livers of Mice

N. C. Sadler[2], V. Mikheev[1], J. G. Pounds[2], R. A. Corley[2] and A. T. Wright[2]. [1]*Battelle Memorial Institute, Columbus, OH and* [2]*Pacific Northwest National Laboratory, Richland, WA.*

Obesity and tobacco smoking are major risk factors for human morbidity and mortality. Each risk factor also produces changes in xenobiotic metabolism and toxicity although the combined effect has not been evaluated. In this study, the interactions between diet-induced obesity (DIO) and P450 function in male C57Bl/6J mice that were raised on standard laboratory diet (5 kcal % fat) vs. high-fat/high-calorie diet (60 kcal% fat) were evaluated following mainstream (MS) and side-stream (SS) cigarette smoke exposures. MS (250 µg/L WTPM) and SS (85 µg/L WTPM) exposures were normalized to the same CO air concentrations. After two weeks of exposure to clean air (control), MS, and SS smoke, multiple P450 activities were analyzed in lung and liver microsomes using an activity-based protein profiling (ABPP) approach. Significant organ-, diet-, and exposure-dependent effects were observed in the function of nearly 30 P450s. For example, hepatic 1A and 2A family P450s were induced and highly active following MS smoke exposures and, to a lesser degree, following SS exposures in standard and DIO mice. Alternately, hepatic 2D and 2E family P450 activities were higher following SS than for MS smoke exposures with greater differences observed in standard vs. DIO mice. Lung P450 activity results also proved to be complicated. As expected, lung 1A1 was not measured in the control, unexposed mice standard and DIO mice whereas activity levels were elevated in following MS and to a lesser degree SS exposures. Interestingly, DIO mice had higher lung 1A1 expression levels following MS smoke exposure than any other lung P450s. These studies reveal that the interactions between smoking (MS and SS) and obesity significantly alters P450 function that can ultimately affect tolerance and/or clearance of therapeutic drugs, xenobiotics, and potentially the carcinogenic potential of tobacco smoke constituents. Funded by NIEHS Grant No. U54 ES016015.

3044 Metformin Prevents Cigarette Smoke-Induced Nrf2 Downregulation and BBB Endothelial Toxicity: A New Therapeutic Use of an Old Drug?

S. Prasad[1], R. K. Sajja[1] and L. Cucullo[1,2]. [1]*Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Amarillo, TX and* [2]*Texas Tech University Health Sciences Center, Center for Blood Brain Barrier Research, Amarillo, TX.*

Metformin, a first line anti-diabetic drug, is recently shown to have anti-oxidant effects by promoting activation of Nrf2 dependent redox homeostasis. Previously, we demonstrated that cigarette smoke exposure (CSE) induces a significant increase in endothelial oxidative stress, leading to blood-brain barrier (BBB) dysfunction. Since Nrf2 impacts BBB integrity, this study examined the effects of CSE on Nrf2 signaling and explored the protective effect of metformin against CSE-induced BBB endothelial oxidative stress. Primary cultures or immortalized mouse brain microvascular endothelial cells (bEnd.3) were exposed to 5% CSE (model of mainstream smoke exposure) with/without metformin pretreatment (10μM) for 24 hours. In addition, male C57BL/6J mice (n=4/group) were exposed via direct inhalation to oxygenated air or cigarette smoke (CS) for 4 weeks in accordance with ISO/FTC protocol. Smoke exposure levels were confirmed by measuring plasma cotinine every week by RIA. Western blots of endothelial cell extracts and whole brain homogenates indicated a marked down-regulation of total Nrf2 by CSE/CS, despite a significant increase in Nrf2 nuclear translocation in BBB endothelium. Importantly, our results also provide novel evidence that Nrf2 regulates mitochondrial ABCB10 transporter (implicated in mitochondrial homeostasis and prevention of ROS generation) and CSE reduced ABCB10 expression in BBB endothelium. Pretreatment with metformin *in vitro* restored CSE-induced Nrf2 down-regulation back to control levels. Additional studies are currently in progress to elucidate the effects of metformin on CS-induced endothelial oxidative stress and BBB impairment *in vivo*. In summary, our data demonstrate the anti-oxidant potential of metformin to mitigate cigarette smoke-induced BBB endothelial oxidative stress by activation of Nrf2 signaling or blocking its down-regulation.

3045 N-3 Polyunsaturated Fatty Acids Protect Against Cigarette Smoke-Induced Vascular Dysfunction

E. F. Wiest[3], M. T. Walsh[3], M. Rothe[1], W.-H. Schunck[2] and M. K. Walker[3]. [1]*Lipidomix GmbH, Berlin, Germany;* [2]*Max-Delbrück Center for Molecular Medicine, Berlin, Germany and* [3]*Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

In cigarette smokers, endothelial dysfunction, as measured by flow-mediated dilation, precedes cardiovascular disease (CVD) and can be improved by supplementation with n-3 polyunsaturated fatty acids (PUFAs). We hypothesized that the loss of nitric oxide (NO)-dependent vasodilation in cigarette smoke (CS)-exposed mice would be prevented by n-3 PUFA supplementation. Methods: Four month old C57BL/6 mice were fed a chow or n-3 PUFA diet for 8 weeks and then exposed to mainstream CS (~300 TPM/m3) or filtered air for 5 days (n=7/group). Mesenteric vessels were isolated, pre-constricted with U46619 (a thromboxane analog), and then dilated with Acetylcholine (ACh) (10-9 - 10-4M) or by stepwise increases in pressure (0-40mmHg), resulting in increases in flow, ± LNNA (NO synthase inhibitor). Results: CS-exposed mice on a chow diet had impaired flow-mediated dilation (FMD), compared to air exposed mice (air, 100±4.8%; CS, 45.3±4.8%, $p<0.05$). The impaired dilation was restored in CS-exposed mice by an n-3 PUFA diet (air, 117.0±8.2%; CS, 95.5±7.3%). There was no difference in total ACh-mediated dilation. However, NO-dependent dilation differed between ACh and FMD. ACh-mediated, NO-dependent dilation was restored in mice on an n-3 PUFA diet (Chow: air, 53.0±4.1%; CS, 25.8±4.1%, $p<0.05$; n-3 PUFA: air, 46.6±5.9%; CS, 56.6±5.3%), while FMD-mediated, NO-dependent dilation was not (Chow: air, 61.1±4.8%; CS, 16.2.8±4.8%; n-3 PUFA: air, 34.0±8.3%; CS, 19.6±8.9%). Although there was no difference in NO-dependent dilation as measured by FMD, NO-independent dilation was increased in mice exposed to CS on a n-3 PUFA diet (Chow, 29.1±4.8%; PUFA, 58.1±8.9%). There was no difference in ACh-mediated NO-independent dilation. Conclusions: Impaired ACh-mediated dilation and FMD in CS-exposed mice can be normalized by consumption of n-3 PUFAs. Specifically, n-3 PUFAs increase NO-independent FMD and NO-dependent ACh-mediated dilation in CS-exposed mice. Dietary n-3 PUFAs may be a therapeutic approach to prevent or delay onset of CVD in smokers and prevent endothelial dysfunction.

3046 Volatile Organic Compound Emissions of Cigarette Smoke of 11 Different Cigarette Brands

C. G. Pauwels[1,3], F.-J. Van Schooten[3], A. W. Boots[3], A. Opperhuizen[2,3] and R. Talhout[1]. [1]*National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands;* [2]*Netherlands Food and Consumer Product Safety (NVWA), Utrecht, Netherlands and* [3]*Toxicology and Pharmacology, Maastricht University, Maastricht, Netherlands.*

Introduction: Cigarette smoke includes more than 7000 chemicals, known to cause tobacco-related diseases. Inhaled Volatile Organic Compounds (VOCs) are partly exhaled again and a fraction is taken up by the lungs leading to internal doses that may have various biological effects. Smokers differ in their smoking behavior and different inter-individual and intra-individual puffing profiles can be observed. Furthermore cigarette brands produce a different VOC emission when smoked, that in combination with varying puffing regimes determine the exposure of the smoker to VOCs. We performed laboratory smoking machine experiments to determine the exact emitted VOCs emissions from different brands of cigarettes under varying puffing regimes. Normally, during laboratory machine smoking experiments the ISO puffing regime is used. However, smoking topography data shows a more intense human smoking behavior. Therefore, a more intense regime, such as the Health Canada Intense (HCI) is also tested. The research question is how smoking regimes influence the VOC emissions from different cigarette brands and whether the outcome is relevant to estimate the actual smokers' exposure to VOCs? Methods: 11 cigarette brands differing in tar, nicotine and carbon monoxide (TNCO) levels, popularity and cigarette manufacturer were machine smoked, following 2 regimes (ISO and HCI), with and without taping the ventilation holes. Blocking ventilation holes imitates the smoker holding and rolling the cigarette between their fingers. VOCs in cigarette smoke were trapped on a Cambridge filter pad and carboxen cartridge. After dissolving in methanol/CS2, the content is determined with GC/MS. Results: Blocking ventilation holes or higher puffing volume increases the VOC emissions of cigarette smoke. When combined, the VOC emission increases even more, especially in the ISO low-TNCO brands. Discussion: Laboratory machine smoking predicts that smokers are exposed to higher levels of VOCs when the brand is intensely smoked, and ventilation holes are blocked. Future research includes human experiments measuring individual smoking profiles and VOCs in exhaled breath.

3047 Cigarette Smoke Dampens Anti-Viral Responses by Disrupting the TLR3/IFN Axis in Lung Epithelial Cells

P. F. Duffney, C. E. McCarthy, T. H. Thatcher, R. P. Phipps and P. J. Sime. *University of Rochester, Rochester, NY.*

Cigarette smoking is the most common intentional insult to the lungs and is a risk factor for the development of many pulmonary diseases. Smokers have an increased prevalence and severity of bacterial and viral infections which suggests a dysregulated innate immune response. The mechanism by which cigarette smoke impairs innate immunity is not fully understood. Epithelial cells are the first line of defense against inhaled stimuli and generate inflammatory and antiviral signals that are key in limiting viral infection. We hypothesize that cigarette smoke dysregulates key antiviral and inflammatory signals in airway epithelial cells which leads to increased susceptibility to viral infection. Primary human lung small airway epithelial cells were grown on culture inserts and exposed to whole cigarette smoke at the air-liquid interface for 60 minutes. Following cigarette smoke, cells were treated with the viral mimetic, polyinosinic-polycytidylic acid (poly I:C). Supernatants were harvested to measure the production of the interferon stimulated protein CXCL10 (IP-10). Levels of interferon beta (IFNβ) and interferon lambda (IFNλ) mRNA were measured 6 hours post-poly I:C treatment and analyzed by qPCR. Interferon bioactivity in culture supernatants was assessed using HEK cells transfected with an interferon stimulated reporter element (ISRE)-luciferase construct. Association of the antiviral signaling molecules TNF receptor associated factor (TRAF) 3 and TANK-binding kinase (TBK) 1 was determined by co-immunoprecipitation. TLR3 protein was analyzed by western blot. Cigarette smoke inhibited poly I:C stimulated production of IP-10 and upregulation of IFNβ and IFNλ, which correlated with reduced ISRE activity in culture supernatants. Co-precipitation of TRAF3 and TBK1 was seen in poly I:C stimulated cells but not in cells with prior cigarette smoke exposure. TLR3 western blots suggest that cigarette smoke alters the ability of TLR3 protein to be activated by proteolytic cleavage. These data suggest that cigarette smoke dampens antiviral responses by disrupting the TLR3/IFN axis which may contribute to the greater susceptibility of smokers to pulmonary viral infection.

3048 The Effect of Electronic Liquids (E-Liquids) on Human MG-63 Osteoblast-Like Cells

M. M. Brown and S. J. Heggland. *Biology, The College of Idaho, Caldwell, ID.*

Electronic cigarettes (e-cigs) are rapidly gaining worldwide popularity as a healthier alternative to traditional tobacco products. The potential risks that e-cigs impose on human health are currently under investigated. There are also indirect consequences of e-cig use including recent reports of children becoming seriously ill due to drinking electronic liquid (e-liquid). The unidentified health risks associated with e-cig use combined with the lack of federal regulations on production standardization point to the need for e-cig research. Since tobacco use is a well-known risk factor for bone-related diseases, we are interested in the effect of e-cigs on bone-forming osteoblasts. We hypothesize that exposure to e-liquid can impair osteoblast function. Human osteoblast-like MG-63 cells were exposed to different concentrations of commercially available e-liquids with or without nicotine either un-vaporized or vaporized for 24 or 48 hours. Cell viability was assessed using an MTT assay. Alkaline phosphatase (ALP) activity, a widely used marker for osteoblast activity, was also measured. Un-vaporized e-liquids were found to significantly reduce cell viability in a dose-dependent manner. This reduction was exacerbated with flavorings agents. However, vaporized e-liquid had variable effects on cell viability dependent on flavoring and nicotine concentration. Specifically, sweet melon flavoring with nicotine uniquely exhibited a proliferative effect. Therefore, we were interested in sweet melon's effect on osteoblast function. Ongoing studies are evaluating the effect of sweet melon, as well as other e-liquids, on ALP activity. Overall, this is the first study to demonstrate e-liquids can have a direct impact on osteoblasts. This study provides insight into the effect of electronic cigarettes on bone health. Research funded by NIH grant #P20GM103408.

3049 Evaluation of CYP3A4 Inhibition, Induction and Hepatotoxicity Using DMSO-Treated Human Hepatoma HuH-7 Cells

Y. Liu[2], T. J. Flynn[2], M. Xia[1] and P. L. Wiesenfeld[2]. [1]*National Institutes of Health, Bethesda, MD and* [2]*US FDA, Laurel, MD.*

A human hepatoma cell line (HuH-7) was evaluated as a metabolically competent cell model to investigate cytochrome P450 3A4 (CYP3A4) inhibition, induction, and hepatotoxicity. First, CYP3A4 gene expression and activity were determined in HuH-7 cells under three culture conditions: one-week culture, three-week culture, or 1% dimethyl sulfoxide (DMSO) treatment. HuH-7 cells treated with DMSO for two weeks after confluence expressed the highest CYP3A4 gene expression and activity compared to the other two culture conditions. Furthermore, CYP3A4 activity in DMSO-treated HuH-7 cells was compared to that in a human hepatoma cell line (HepG2/C3A) and human bipotent progenitor cell line (HepaRG), which yielded the following ranking: HepaRG > DMSO-treated HuH-7 >> HepG2/C3A cells. The effects of three known CYP3A4 inhibitors were evaluated using DMSO-treated HuH-7 cells. CYP3A4 enzyme inhibition in HuH-7 cells was further compared to human recombinant CYP3A4, indicating similar potency for reversible inhibitors (IC50 values within 2.5 fold), but different potency for the irreversible inhibitor. Next, induction of CYP3A4 activity was compared between DMSO-treated HuH-7 and HepaRG cells using two known inducers. DMSO-treated HuH-7 cells yielded minimal CYP3A4 induction compared to that in the HepaRG cells after 48h treatments. Finally, the cytotoxicity of five known hepatotoxicants was evaluated in DMSO-treated HuH-7 cells, HepG2/C3A, and HepaRG cells, and significant differences in cytotoxic sensitivity were observed. Overall, DMSO-treated HuH-7 cells are a valuable model for medium- or high-throughput screening of chemicals for CYP3A4 inhibition and hepatotoxicity. Keywords: HuH-7 cells; DMSO; CYP3A4; inhibition; induction; hepatotoxicity Category: Alternatives to Mammalian Models

3050 Picking Cell Lines for High-Throughput Transcriptomic Toxicity Screening

R. W. Setzer and R. S. Thomas. *National Center for Computational Toxicology, US EPA, Research Triangle Park, NC.*

High throughput, whole genome transcriptomic profiling is a promising approach to comprehensively evaluate chemicals for potential biological effects. To be useful for *in vitro* toxicity screening, gene expression must be quantified in a set of representative cell types that captures the diversity of potential responses across chemicals. The ideal dataset to select these cell types would consist of hundreds of cell types treated with thousands of chemicals, but does not yet exist. However, basal gene expression data may be useful as a surrogate for representing the relevant biological space necessary for cell type selection. The goal of this study was to identify a small (< 20) number of cell types that capture a large, quantifiable fraction of basal gene expression diversity. Three publicly available collections of Affymetrix U133+2.0 cellular gene expression data were used: 1) 59 cell lines from the NCI60 set; 2) 303 primary cell types from the Mabbott et al (2013) expression atlas; and 3) 1036 cell lines from the Cancer Cell Line Encyclopedia. The data were RMA normalized, log-transformed, and the probe sets mapped to HUGO gene identifiers. The results showed that <20 cell lines capture only a small fraction of the total diversity in basal gene expression when evaluated using either the entire set of 20960 HUGO genes or a subset of druggable genes likely to be chemical targets. The fraction of the total gene expression variation explained was consistent when evaluated using either linear combinations of genes and cell lines or by centroids of clusters of cell lines. Alternatively, only ten cell lines are required to capture the diversity in the fraction of genes that are highly expressed in at least one of the cell lines. This analysis demonstrates the challenges in profiling the entire genome based on a limited number of cells. Future efforts are needed to explore alternative approaches to selecting representative cell lines that adequately cover relevant biological space for high-throughput transcriptomic toxicity screening. This abstract does not necessarily reflect US EPA policy.

3051 Developing a Mitochondrial Toxicity Pathway-Based Framework for Chemical Safety Assessment: A Case Study of PGC-1α-Mediated Network Perturbation with Doxorubicin

H. Yuan[3], Q. Zhang[1], J. Guo[3], T. Zhang[3], J. Li[2], P. Carmichael[2], A. White[2], C. Westmoreland[2], J. Zhao[3] and S. Peng[3]. [1]*Hamner Institute for Health Sciences, Research Triangle Park, NC;* [2]*The Unilever Safety and Environmental Assurance Centre, Sharnbrook, Bedford, United Kingdom and* [3]*Institute of Disease Control and Prevention, Academy of Military Medical Sciences, Beijing, China.*

Mitochondrial toxicity is increasingly implicated in the adverse effects of many failed drugs and chemicals. Pathway-based safety assessments for chemicals with mitochondrial toxicity require two critical steps: to identify relevant biochemical (toxicity) pathways mediating mitochondrial homeostasis and then to derive biological pathway altering doses (BPADs) based on pathway perturbations. In this work, we identified that peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α)-mediated transcriptional network plays central roles in the regulation of mitochondrial oxidative stress and biogenesis. As a case study, we conducted *in vitro* studies to measure redox, gene and mitochondrial functional biomarkers in the human myocardial cell line AC16, exposed to a drug, doxorubicin (Dox) with known cardiac side effects *in vivo*. Our biomarker profiles collectively indicate a well-defined tipping point, suggesting that a BPAD for Dox for mitochondria stress can be determined. *In vitro* data demonstrates adaptive induction of PGC-1α regulated downstream genes, including NRF-1, MnSOD, UCP2 and COX1, concurrent with negligible changes in superoxide, ATP and cytotoxicity; whilst above the tipping point, at doses resulting in saturation of the PGC-1α pathway, cellular adversity is evident in elevated superoxide and suppressed ATP levels. Finally, we developed a computational model to simulate the PGC-1α-mediated pathway network comprising multiple negative feedback loops that underlie redox and bioenergetics homeostasis. The model recapitulated our experimental outcomes, suggesting that saturated induction of the PGC-1α-mediated gene network underpins this BPAD. It further predicted that silencing PGC-1α compromises the adaptive function of the pathway, leading to disruption of mitochondria and cytotoxicity at lower Dox doses, which was verified by our study. This study illustrates that combining computational simulation and *in vitro* assays anchored to pathway information offers a sound approach to identify BPADs with increased confidence.

3052 Challenges in Alternative Methods: Example of the Steroidogenesis Assay

A. Verlohner, T. Ramirez, V. Strauss, R. Landsiedel and B. van Ravenzwaay. *Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

The steroidogenesis assay is a method to identify substances that affect the production of androgen or estrogen steroid hormones. It uses the human adreno-carcinoma cell line NCI-H295R which produces all steroid hormones found in the adult adrenal cortex and the gonads. The method was adopted as OECD TG 456 in 2011 and is part of the *in vitro* testing strategy of the endocrine disruptor screening program (US EPA). Four years ago we established the method in our laboratories and report here on 2 important challenges. The method requires a proficiency test consisting with 5 positive and 1 negative control, the latter being the human chorionic gonadotropin (HCG). We noted that the required

amounts of HCG are difficult to obtain in Europe and that the batch that we received was contaminated with estrogen-conjugates, making impossible the reliable assessment of the effects of this test substance on the system. The second challenge occurred after several years of successful use of this assay when we encountered a significant "background" presence of testosterone in a charge of the serum proposed by the OECD TG 456. We found out that the used serum presented 60x higher testosterone concentrations than normal, making impossible to meet quality control criteria for this assay. At that time no other such serum was available, therefore we tried several alternatives, e.g. stripping of the serum with charcoal, the use of hormone-free FBS serum and as last instance the use of regular FBS serum. Cells were grown under all these conditions and quality control plates were prepared in all cases. The best condition was a combination of the use of serum during the maintenance of the cells and then replaced by FBS serum during test substance exposure. Under these conditions the quality criteria were meet and the hormone levels of control cells were as within the historical control range. We were not informed about the change in the serum and we can only speculate that other laboratories may also have been affected. Lessons learned: small changes may have profound effects on the results of *in vitro* testing, and not always are such changes foreseeable. Performance standards and historical control values are as important in *in vitro* assays as they are in *in vivo* studies.

PS 3053 Characterization of Human Proximal Tubule Cells for Investigating Drug-Induced Nephrotoxicity

K. C. Wu, M. Singer and M. Otieno. *Investigative Toxicology, Janssen R&D, Spring House, PA.*

The kidney proximal tubule is a major target for drug-induced toxicity due to its ability to transport and potentially accumulate xenobiotics. It is also the primary site of injury for drugs that interfere with cellular energetics and/or mitochondrial function. The present study aimed to characterize human primary renal proximal tubule cells (HPRTC) to assess its utility for mechanistic investigation of drug induced nephrotoxicity. HPRTC could be cultured for up to 11 days and lot (VWQ) was identified to have the most optimal profile for expression of major renal xenobiotic transporters and drug metabolism enzymes. However, all lots of HPRTC tested did not express organic anion transporters (OATs). HPRTC was evaluated with 18 tool nephrotoxicants (human/animal, and as expected most compounds known to be transported by OATs were not toxic in the *in vitro* assay. Endpoints included cell viability, mitochondrial ATP depletion, and mitochondrial respiration using the Seahorse platform. Cisplatin, cadmium chloride, pentamidine, and amphotericin B were cytotoxic at IC50s that were comparable to maximum drug concentrations reported in human kidneys. There was a left shift in IC50 for mitochondrial ATP depletion for adefovir, dipivoxil, pentamidine, potassium dichromate, and amphotericin B compared to cytotoxicity identifying mitochondria as a potential target organelle for nephrotoxicity by these compounds. This was further confirmed using the Seahorse platform where mitochondrial respiration was inhibited or uncoupled by these compounds In conclusion, HPRTC express major renal xenobiotic transporters and drug metabolism enzymes except OATs, are sensitive to some nephrotoxicants at pathological relevant concentrations, and can also be used to study mitochondrial-mediated toxicity. Cell lines with full spectrum of uptake transporters as well as sensitive endpoints are needed to allow the prediction of drug-induced proximal tubular toxicity at early pre-clinical stages.

PS 3054 Mode of Action Differentiation and Elucidation with Metabolomics *In Vitro*

T. Ramirez[2], A. Strigun[1], W. Mellert[2], H. G. Kamp[2], T. Walk[1] and B. van Ravenzwaay[2]. [1]*metanomics GmbH, Berlin, Germany and* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

The development and application of alternatives to animal testing has considerably increased in the last years but is still mostly limited to topical applications. The most relevant toxicological endpoints for which alternative methods need to be developed are systemic (organ), developmental and reproductive toxicity. Although they represent a major challenge, progress can be made by applying innovative technologies, which allow for multi-parameter assessment as well cell providing information on the biological changes in the cells. In this context, metabolomics *in vitro* is a novel approach that can enable the identification of toxicity modes of action for systemic (organ) toxicity, when applied in combination to *in vitro* organ models. Herein, we report on the establishment of a highly reproducibility HepG2-based system which we developed and validated over a period of more than 3 years using both supernatant (140 metabolites identified) and intracellular metabolome analysis (238 metabolites). We also report on the success to identify, with high specificity different modes of action of liver toxicants (e.g. liver enzyme induction and inhibition, liver toxicity and peroxisome proliferation). The results obtained demonstrate that the liver active substances (more than 20 compounds analysed) produce differential metabolome profile which can be grouped by mode of action. By investigating more in detail the relation among the metabolites changed by the peroxisome proliferators we can observe a clear changes in those metabolites related to the beta-oxidation of unsaturated fatty acids, which correlates well to the mechanism of this class of test substances in the *in vivo* situation. The present results strongly suggest that this technology is ready for its application in toxicology to support the identification of systemic (organ) toxicity.

PS 3055 Effect of Reactive Oxygen Species Quenchers on *In Vitro* Photosensitization Evaluation

A. Toyoda[1,2] and H. Itagaki[1]. [1]*Yokohama National University, Yokohama, Japan and* [2]*Quality Research Department, POLA Chemical Industries,INC., Yokohama, Japan.*

There are two mechanisms underlying photoallergic dermatitis: prohaptens become haptenized by ultraviolet (UV) irradiation before bonding covalently with a protein, or photohaptens bond covalently to a protein while simultaneously undergoing partial photodecomposition in response to UV irradiation. Most photoantigens have the characteristics of photohaptens1).Reactive oxygen species (ROS) assays have recently been applied to evaluate photosafety, and it is considered that the ROS in photoallergens are involved in photohapten and photodecomposition. We investigated the effect of ROS on the *in vitro* photosensitization test method using ROS quenchers. Photo-h-CLAT determines the expression of CD86 and CD54 on THP-1 human cell-line cells after the test substance and cells are exposed to a constant level of UV light for 24 hours. Prior to UV irradiation, different quenchers were added to the cells before exposing the cells to the test substance. We evaluated changes in CD86 and CD54 expression in response to the addition of quenchers and assessed the effect of the quenchers in reducing toxicity. When histidine, which has a quenching effect on singlet oxygen, was added to the photoallergens chlorpromazine and bithionol, a decrease was observed in CD86 and CD54 expression under UV irradiation. This quenching effect was also seen in other ROS quenchers, demonstrating that ROS were involved in photoallergenicity. Based on these results, we intend to elucidate the mechanisms underlying photoallergenicity in order to minimize photoallergic reactions. We also intend to improve the sensitivity of the photo-h-CLAT assay. References: 1) Tokura. Y, Expert review of dermatology, 2009, 4(3), 263-270 2) Hoya. M, et al., Toxicology *in vitro*, 2009, 23, 911-918

PS 3056 Evaluation of Two Routinely Used Cell-Based Screening Assays for Neurotoxicity

A. H. Heussner[2], I. Richter[1,2] and D. R. Dietrich[2]. [1]*Cawthron Institute, Nelson, New Zealand and* [2]*Human and Environmental Toxicology, University of Konstanz, Konstanz, Germany.*

There is growing need for rapid elucidation of the neurotoxic potential of natural substances, bulk chemicals or drugs. A prominent example are marine biotoxins which pose an increasing threat to human health and economy due to the expanding endemic areas of toxin producers and increased global marketing of fish, shellfish and crustaceans. Traditionally, rodent bioassays (RBA) were widely established for toxicity evaluation, however RBA are no longer accepted within the EC. Alternatives include amongst others screening assays with neuroblastoma cell lines of murine (Neuro-2a) or human (SH-SY5Y) origin and general cytotoxicity determined via MTT reduction. Despite the simple nature of the latter assays, published data revealed an enormous variation in assay parameters used: cell culturing, cell exposure and toxicity endpoints. Therefore, the comparison of published data is nearly impossible and does not instill confidence in the neurotoxicity data generated. Thus the aim of the current study was to determine the impact of assay parameters e.g. cell culture conditions (media, serum content), cell differentiation procedures (differentiation inducing agents, differentiation time) and exposure regimes to test substances (concentration range used, cell number, exposure time, solvents) on the results of neurotoxicity toxicological screening with Neuro-2a and SH-SY5Y cells. The neurotoxic compounds tested included cadmium chloride, ouabain, veratridine, nicotine, carbamazepine, N-butyl-p-aminobenzoate and brevetoxins. Furthermore, to compare results with those obtained with the neuroblastoma cell lines non-neuronal cells (NRK-52E) were used. The results indicate that

the sensitivity to neurotoxins varies, largely depending on the mode of action of the toxin applied and what assay parameter was changed. Cell differentiation state was the most important factor. The results confirmed the initial assumption that the choice of the *in vitro* model as well as the testing conditions is crucial for the outcome.

3057 Use of Metabolomics *In Vitro* to Estimate an Acceptable Daily Intake Ranking

W. Mellert[2], T. Ramirez[2], A. Strigun[1], H. G. Kamp[2], T. Walk[1] and B. van Ravenzwaay[2]. [1]*Metanomics GmbH, Berlin, Germany and* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

The use of *in vitro* technologies for screening purposes and gaining mechanistic insights about the toxicity of new compounds has considerably increased in the last years. Over the last 3 years we have successfully build a system combining *in vitro* cell culture with metabolome analysis (analysing 238 intracellular metabolites), that is stable and reproducible and with which we can identify different modes of action of liver toxicants. Using this experimental set up, we investigated if the quantitative information obtained in such experiments can be used to rank test substances according to their metabolomics NOEC/LOEC with a ranking of the acceptable daily intake (ADI). For this purpose, we used HepG2 cells treated with 10 different compounds (with known ADI) for 48h at 5 different concentrations. After exposure, LC-MS/MS analytics was applied to measure intracellular metabolomics. The number of changes between independent control groups (5 groups, 80 samples) was used to determine normal variation. This value was then used as the false discovery rate to determine the point of departure for the start of a test substance related increase in metabolome change in the dose response curve for each compound. These NOEC/LOEC concentrations were then compared with the ADI values for these compounds. With this approach we could distinguish correctly between high (> 1 mg/kg bw), low (0,01 ADI 0.01 ADI < 0.02) but failed in 2 cases for ADI values > 0.02. The results of this proof of concept work suggest that *in vitro* 'omics technologies such as metabolomics could support a first rough estimation of ADI in a fast an efficient fashion. We are expanding the data set and will integrate this approach with biokinetic models to enhance the accuracy of the ADI prediction model.

3058 Enabling Chemical Grouping by Applying Metabolomics *In Vitro*

B. van Ravenzwaay[2], T. Ramirez[2], A. Strigun[1], W. Mellert[2], H. G. Kamp[2] and T. Walk[1]. [1]*Metanomics GmbH, Berlin, Germany and* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany.*

Chemical grouping and read-across are important ways to reduce animal testing, provided that the grouping procedure is convincing and transparent. New technologies with multi-parameter analysis can potentially be of use to improve chemical grouping by taking into account the biological response. We report on a proof of concept study in which we combined metabolomics with *in vitro* toxicology by evaluating the metabolome (analysing 238 intracellular metabolites) of HepG2 liver cells using GC- and LC-MS/MS. For this purpose, HepG2 cells were exposed over 48 hrs to the racemic mixture and optical active isomer of mecoprop, dichlorprop and dimethenamid. Our data demonstrate that, similarly to the *in vivo* situation, the toxicological equivalence between the racemate and their corresponding optical active form can be shown in this *in vitro* set up for all 3 compounds. Moreover, we were also able to identify the similarities of the metabolome of mecoprop and dichlorprop (for both racemate and pure isomer). Both compounds also show *in vivo* a very similar toxicological profile. Finally we can show that the *in vitro* metabolome of dimethenamid, is dissimilar to that of mecoprop and dichlorprop, corresponding well to the *in vivo* differences in toxicity. It should be noted here, that all 3 compounds have an effect on the liver and that the differences in their toxicological profiles are not very profound. This study is an initial proof of concept indicating that chemical grouping could be also achieved by means of *in vitro* models used in combination with innovative technologies, such as metabolomics.

3059 Comparison of Liver Cell Models to Identify Chemically Induced Cytotoxicity

R. Faris[2], Y. Hong[2], J. Liu[2], R. Zuo[1], F. Li[2], M. Andes[1] and C. J. Patten[1]. [1]*Corning Life Sciences, Corning Inc, Bedford, MA and* [2]*Science and Technology, Corning Inc, Corning, NY.* Sponsor: C. Crespi.

Unforeseen drug-induced liver injury (DILI) is one of the primary reasons for aftermarket black box warnings and removal of drugs. As a result the FDA recently recommended the development of novel tools and cell-based assays to more accurately identify DILI. Primary human hepatocyte culture is presently the gold standard model for studying the ADMET properties of drug candidates. However, due to large lot-to-lot variations and limited lot size of primary hepatocytes, significant time and resources are spent on prequalifying primary hepatocytes. Our laboratory recently developed Corning® HepatoCells; single-use, cryopreserved cells derived from normal primary hepatocytes from a nine year old female donor. In the present investigation, HepatoCells, primary human hepatocytes from two donors and HepaRG were employed to screen a compound library that contained toxic and non-toxic chemicals (Enzo Screen-Well® Hepatotoxicity Library). Liver cells were cultured on Corning® Biocoat Type I Collagen plates with a Matrigel® overlay using the manufacturer's recommended hepatocyte medium. On culture day 5 liver cells were pretreated with a panel of 238 compounds (100uM). Cell viability was measured 48 hours after treatment using the Promega CellTiter GLO assay and data was normalized to DMSO controls. Cytotoxicity was defined as a 50% reduction in cell viability. Linear regression analysis of hepatotoxicity data revealed that the compound toxicity profile of HepatoCells more closely modeled the behavior of primary and parental donor primary hepatocytes (R2=0.68) than HepaRG™ (R2=0.46). Thirty chemicals exhibited greater than 90% cytotoxicity towards both primary hepatocytes and HepatoCells; of these, 12 were not toxic in HepaRG (e.g. >75% viability). Of note, HepatoCells and parental hepatocytes were more sensitive than HepaRG to the cytotoxic effects of several known estradiol receptor ligands (e.g. tetrachlorobisphenol A, nordihydroguaiaretic acid, diethylstilbestrol). Our results suggest that Corning® HepatoCells retain the complement of hepatic drug metabolism enzymes and transporters which closely models the behavior of primary cells, and therefore, may be used as a surrogate to screen for cytotoxicity early in the drug discovery process.

3060 Assessment of an *In Vitro* Human Intestinal Epithelial Cell Model for Evaluation of Protein Cytotoxicity

C. Zimmermann[4], B. Hurley[1,3], A. Eaton[3] and B. Delaney[4]. [1]*Department of Pediatrics, Harvard Medical School, Boston, MA;* [2]*Global Regulatory Science, DuPont Pioneer, Johnston, IA;* [3]*Mucosal Immunology Laboratory, Massachusetts General Hospital, Boston, MA and* [4]*Scientific Affairs, DuPont Pioneer, Johnston, IA.*

An *in vitro* testing system employing polarized human intestinal epithelial cells (IEC) that model the gastrointestinal (GI) barrier was evaluated as a potential tool to identify potential for adverse effects following exposure to proteins that are similar in function (i.e. insecticidal proteins) or classification (i.e. intractable proteins) to proteins that could potentially be incorporated into plants to improve agronomic qualities or impart insecticidal activity. Proteins tested included the following intractable proteins: bacteriorhodopsin (transmembrane); human c-MET (signaling/transmembrane); follistatin (signaling); and activating transcription factor 2 (transcription factor). An insecticidal protein from the Australian funnel web spider (Ω Hexatoxin-Hv1a) was also tested. No effects were observed on cytotoxicity, barrier integrity or permeability, or inflammation for any of the intractable or insecticidal proteins tested. These results indicate the potential of this *in vitro* assay to predict the safety of proteins similar to those that may be genetically engineered into plants to impart insecticidal activity or improve agronomic performance.

3061 DILI Prediction Connecting Organ Level Response to Cellular Mechanism

S. Das[2], O. Irrechukwu[1], R. Kumar[2], S. Krzyzewski[1], V. R. Zurawski[1], J. K. McGeehan[1] and K. Subramanian[2]. [1]*Hepregen Corporation, Medford, MA and* [2]*Strand Life Sciences Pvt. Ltd, Bangalore, India.*

Late stage compound withdrawal due to hepatotoxicity is associated with limitations in the existing screening methodologies during drug discovery. A drug discovery program needs to either fail a compound early or understand whether the mechanisms underlying the failure can be overcome. To achieve both these goals, we combined two DILI pre-

diction technologies, Hepatopac® and Heptox, to assess the DILI potential and identify the driver mechanisms for acetaminophen, amiodarone, and tamoxifen. Hepatopac identified ATP and GSH depletion caused by APAP and a concomitant reduction in urea and albumin levels upon 5 days of treatment and Heptox identified the processes that caused the effect: mitochondrial membrane depolarization, increase in ROS generation and inhibition in GSH synthesizing GR and γ-GCS enzymes leading to the ATP & GSH depletion. Similarly, for both amidarone and tamoxifen, known to cause steatotic DILI, Hepatopac identified reduction in the plasma biomarkers urea and bilirubin, while the ATP and GSH seemed unaffected. Heptox predicted that alterations in fat metabolism enzymes, FAS and CPT1, fatty acid transporter and microsomal tri-glyceride transfer protein (MTTP), would cause elevated levels of cellular triglycerides, leading to compromised organ function reflected as a change in plasma biomarkers along with an increase in ALT levels. Combining these two technologies provides both an accurate screening mechanism for hits and leads along with a mechanistic rationale that facilitates lead selection and optimization.

3062 Human BJ Fibroblasts as an Alternative to Mouse Balb/c 3T3 Fibroblasts *In Vitro* Neutral Red Uptake Assay

T. Heinonen, T. Toimela and M. Mannerström. *School of Medicine, University of Tampere, University of Tampere, Finland.* Sponsor: K. Savolainen.

BALB/c 3T3 NRU cytotoxicity assay (OECD Guidance Document 129) is recommended for estimating the starting doses for acute oral systemic toxicity tests. Mouse BALB/c 3T3 fibroblasts or normal human epidermal keratinocyte NHK cells are used in this method. Mouse BALB/c 3T3 fibroblasts assay is most commonly used. We have transferred to and validated BALB/c 3T3 NRU assay in our laboratory for routine use to study acute cytotoxicity. Subsequently we have investigated human BJ fibroblasts, which are comparable with BALB/c 3T3 cells in terms of cost, availability and ease of culturing, as the alternative cells in the NRU assay. The NRU assay was performed identically with both cell types using a set of reference chemicals of sodium dodecyl sulfate (SDS), acryl amide, oxytetracycline dihydrate, bisphenol A and acetone. Our results showed marked differences between the human and mouse fibroblasts in the sensitivity to tested chemicals. The IC50 value of SDS (which is used as positive control in NRU assay) was lower in human BJ fibroblasts (mean of 52 µg/ml) than in mouse BALB/c 3T3 fibroblasts (mean of 86 µg/ml). Also other chemicals studied gave divergent results to both directions; human fibroblasts were more sensitive than mouse fibroblasts to acryl amide and oxytetracycline dihydrate, with IC50 values of 59.4 and 115.40 µg/ml for acryl amide, and 130.4 and 173.40 mg/ml for oxytetracycline dihydrate, respectively. Acetone, in turn, was more toxic to human than to mouse fibroblasts with IC50 values of 11.9 and 49.4 µg/ml, respectively. Bisphenol A was equally toxic to mouse and human fibroblasts. In conclusion, testing chemicals with both human and mouse fibroblasts would give a case-by-case insight to the potential different susceptibility in cellular toxicity of tested compounds between human and mouse. More comparative research with chemicals with human data are needed to clarify whether human fibroblasts predict human acute toxicity better than the mouse fibroblasts.

3063 Botulinum and Tetanus Neurotoxin-Induced Blockade of Synaptic Transmission in Networked Cultures of Human and Rodent Neurons: Toward a Cell-Based Replacement for the Mouse Lethal Assay

P. M. McNutt. *USAMRICD, Gunpowder, MD.*

The clostridial neurotoxins (CNTs), which include tetanus neurotoxin (TeNT) and seven botulinum neurotoxin (BoNT) serotypes, are the most potent poisons known with estimated human LD50 values as low as 0.1-1 ng/kg. The distinctive clinical manifestations of CNT poisoning arise from an intricate series of interactions with nerve terminal proteins that result in the proteolytic cleavage of soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins and blockade of neurotransmitter release. Currently, there is no cell-based model that replicates *in vivo* synaptic responses to CNT intoxication. Here we show for the first time that intoxication of synaptically active, networked neuron cultures results in production of cleaved SNARE proteins and blockade of synaptic neurotransmission at physiological toxin concentrations, thereby reproducing the molecular and functional endpoints of clinical disease. The generalizability of using synaptic neurotransmission as a readout of intoxication was confirmed in human and mouse stem cell-derived neurons as well as primary rat neurons exposed to each CNT. The sensitivity and specificity of synaptic neurotransmission to intoxication by BoNT/A, /B and TeNT were further evaluated in time- and dose-response studies as well as in assays representing the principal clinical and research purposes of *in vivo* paralysis assays. Our findings confirm that measuring synaptic function offers a novel and functionally relevant approach for the *in vitro* characterization of CNTs, with sensitivities that are equivalent to the mouse lethal assay and ten-fold improved over SNARE protein cleavage assays. Synaptic activity in networked neuron cultures offers the first physiologically relevant, *in vitro* surrogate for neuromuscular paralysis and provides a transformational approach for novel phenotypic screens functionally testing for reversal of intoxication. We propose that confirmation of synaptic activity in cell cultures will significantly reduce the need for terminal animal use in toxin studies as well as improve the relevance of cell-based screening of candidate therapeutics.

3064 *In Vitro* Model Development for Safety Assessment of Acutely Ingested Proteins

S. Wezalis[1], J. Yao[1], J. Roper[1], C. Zimmermann[2], B. Delaney[2] and L. K. Markell[1]. [1]*DuPont Haskell Global Centers, Newark, DE and* [2]*DuPont Pioneer, Johnston, IA.*

Proteins that are known to be toxic to humans and other animals following oral exposure are often resistant to digestion by the target organism with toxicity primarily directed toward intestinal cells or with systemic toxicity following absorption from the gastrointestinal system. Intractable proteins are defined as proteins that cannot be isolated in an active form in the quantities necessary to conduct *in vivo* toxicological testing. *In vitro* models using cultured cells may provide an alternative for these regulatory requirements. In order to develop a model for acute toxicity assessment of proteins, Caco-2 human epithelial intestinal cells were cultured for 21 days on permeable Transwell inserts forming a monolayer resembling the enterocyte lining of the small intestines. Phytohaemagglutinin (PHA) from red kidney bean lectin is resistant to digestion and causes ultrastructural damage in rat intestine. Here, PHA was used to determine the effect on intestinal cells *in vitro*, measuring changes in viability and barrier integrity. Following up to 48 hours treatment, PHA caused a dose-dependent decrease in tight junction integrity causing a significant flux in 4.4 kDa TRITC-dextran to the basolateral compartment compared to media alone and this was accompanied by a 40-50% reduction in TEER measurements. The disruption to the monolayer was not size selective as a significant increase in 70 kDa FITC-dextran flux was observed, although cell viability was not altered. In addition, a method for *in vitro* gastric and intestinal digestion of proteins was developed for application on Caco-2 monolayers. Tight junction and monolayer integrity following treatment with PHA that underwent simulated digestion was evaluated. These results demonstrate that an *in vitro* model may be used to predict acute toxicity *in vivo*, potentially providing weight of evidence for protein safety assessments.

3065 Crosstalk Between Mitochondrial Function, the Epigenome and Gene Expression with Potential Implications to Environmental Health

R. Woychik[1], J. H. Santos[1], O. Lozoya[1], I. Martinez[2], D. Grenet[1], T. Wolfgang[1], T. Wang[1] and N. Chandel[2]. [1]*National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC and* [2]*Department of Medicine, Northwestern University Feinberg School of Medicine, Evanston, IL.*

Mitochondria are organelles well-known for their role in energy production and are the target for many environmental agents. It is also known that metabolites from the mitochondria are co-factors or substrates for the enzymes that modify the epigenome in the nucleus. Whether mitochondrial-derived metabolites are rate-limiting for epigenetic reactions and the extent to which they affect gene expression in the nucleus is poorly understood. In this study we leveraged the use of two cell culture models of chronic or acute mitochondrial dysfunction, in combination with several 'omics' approaches, to define which epigenetic changes are associated with mitochondrial function. The data obtained so far indicate that mitochondrial acetyl-CoA is rate limiting for histone acetylation in the nucleus, but not all acetylated histone lysine residues are affected the same way. itochondrial ROS does not affect histone modifications but rather leads to locus specific DNA hypomethylation. These epigenetic changes appear to be orchestrating changes in a gene expression program that is centered on specific pathway regulators. Taken together, our results reveal that changes in mitochondrial function, which can be modulated by various environmental agents, can lead to changes in the epigenome in a way that may impact the cell biology, development and health outcomes of an organism. This research was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

3066 Evaluating the Inflammatory and Genotoxic Effects of Smokeless Tobacco Using a Human Organotypic Model of Oral Epithelium

M. Bachelor, J. Oldach, B. Breyfogle, P. Hayden and M. Klausner. *MatTek Corporation, Ashland, MA.*

The use of tobacco use has been implicated as a major cause of oral cavity disease leading to thousands of deaths per year. Snus, a smokeless tobacco applied to the oral cavity, has been proposed as a less harmful alternative to smoking although its safety has not been adequately evaluated. The objective of this study was to evaluate the cytotoxic, genotoxic and inflammatory effects of snus using an *in vitro* model of human oral mucosa (EpiOral). EpiOral tissues were exposed topically to 5–25 milligrams of snus or snus (CRP1) extracts for 24-48 hours. Tissues exposed to 5 mg of snus had comparable viability to vehicle treated controls while those exposed to 25 mg displayed approximately a 20% decrease in viability after 24 and 48 hours of exposure as determined by MTT analysis. Histological evaluation revealed hyperchromic staining in tissues exposed to 5 mg of snus at 24 hours post-exposure whereas tissues exposed to 25 mg of snus displayed a significant amount of sloughing of the apical layers. Following exposure, an inflammation-specific cytokine panel was used to analyze markers of inflammation 24 and 48 hours post exposure. Of the cytokines analyzed, a significant increase in IP-10 was observed 24 hours post exposurein tissues exposed to 25 mg of snus or 5–10% snus extract. As a measure of genotoxicity, the presence of γ-H2AX foci was evaluated in exposed tissues. γ-H2AX is a phosphorylated derivative of the H2AX histone and is tightly bound to double strand DNA break sites, therefore serving as a biomarker of genotoxic insult. γ-H2AX foci were readily detected in the apical layer of tissues exposed to 25 mg of snus and 10% snus extract at 24 and 48 hours post treatment. Exposure of snus extracts to EpiOral also revealed significant increases in CYP1A1 (600 fold) and CYP1B1 (40 fold) gene expression. These results demonstrate the utility of this organotypic oral tissue model to evaluate smokeless tobacco product safety.

3067 ROS Generation Produced by HepG2 Cells Exposed to DON, 3-ADON and 15-ADON Individually and Combined

A. Juan-Garcia, R. Cipriano, G. Font and M. Ruiz. *Laboratory of Toxicology, University of Valencia, Valencia, Spain.* Sponsor: G. Font.

Derivate products of mycotoxins can be present in cereals and their byproducts. Several toxic effects have been associated to them. This situates mycotoxins as important health concern for humans and consequently in the food safety. Trichothecenes constitute a family of mycotoxins, where deoxynivalenol (DON) is the major representative. Two of its acetylated products, 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), can co-occur when DON is present in food samples. This study aims to monitoring the reactive oxygen species (ROS) produced after exposing HepG2 cells to DON, 3-ADON and 15-ADON individually and combined. Concentrations ranged from 1.5 to 10 µM for individual exposure, while for binary and tertiary mixtures, the concentration ranged from 0.2 to 1.5 µM for 3-ADON and 15-ADON and, from 0.6 to 4.8 µM for DON. Monitoring was performed at intervals of 5 min up 120 min through the H2DCF-DA (2′,7′-dichlorofluorescin diacetate) probe which detect intracellular oxidants. Individual treatment on HepG2 cells demonstrated that low doses of 3-ADON and 15-ADON increase ROS generation respect to the control up to 120 min; DON increase ROS production respect to the control from 45 up to 120 min after individual mycotoxin exposure, the highest ROS production was observed after DON exposure. The binary (DON + 3-ADON and 3-ADON + 15-ADON) and tertiary (DON + 3-ADON + 15-ADON) combinations at early times of exposure (0 to 30 min) showed significant increase in ROS production respect to the control. Whereas, the binary combination (DON + 15-ADON) from 45 to 120 min increase ROS production respect to the control at the highest dose assayed. The highest ROS production was obtained by combination formed by DON´s metabolites: 3-ADON + 15-ADON. Acknowledgements: This research has been supported by the Spanish Ministry of Science and Innovation (AGL2013-43194-P).

3068 Disruption of Cellular Circadian Rhythm by Neurotoxic Agents Can Be Restored with Antioxidants in Neural Cells *In Vitro*

M. Fang[1], Y. Park[2], H.-G. Kang[2], S.-A. Juan[1] and H. Zarbl[1]. [1]*Environmental & Occupational Medicine, School of Public Health, Rutgers, The State University of New Jersey, Piscataway, NJ and* [2]*Veterinary Drugs & Biologics Division, Animal & Plant Quarantine, An-Yang, Korea, Republic of.* Sponsor: M. Fang.

Accumulating evidence suggests that neurodegenerative diseases and psychiatric disorders are often concurrent with sleep/circadian rhythm disruption and also associated with exposure to environmental toxicants. Our previous studies have demonstrated that some environmental chemicals can alter circadian gene expression, which contributes to disease progression in animal models. Although there is a wealth of information about the behavioral and biological influences on circadian disruption and its consequences for neurological function, there is a scarcity of data on what types of environmental chemicals can lead to disruption of circadian rhythm, the mechanisms by which they mediate these effects, and how these effects will impact cellular function and disease outcomes. To implicate the circadian rhythm in development of alternative testing for neurotoxicity, in this study, we constructed a circadian gene promoter driven destabilized luciferase expression vector (PER2-dLuc) and established an *in vitro* cellular bioluminescence assay in neural cell lines. Using this *in vitro* model, we found that a battery of known neurotoxic agents (e.g., dichlorodiphenyltrichloroethane, DDT) disrupted cellular circadian rhythm. More interestingly, some antioxidants (e.g., n-acetylcysteine) that were known to reduce neurotoxicity can prevent the loss of, or restore a normal pattern of cellular circadian rhythm. Ongoing mechanism studies are expected to elucidate a mechanistic link between circadian disruption and neurotoxic endpoints at different sites of cells. (Supported by 2012 SOT-Colgate Palmolive Grant for Alterative Research and by International Collaboration Fund from the Plant and Animal Quarantine Agency in South Korea).

3069 Use of a New Human Small Intestinal Tissue Model to Screen Drug Induced Gastrointestinal Toxicity

S. Ayehunie[2], M. Wagoner[1], H. Barthlow[1], C. Scott[1], Z. Stevens[2], T. Landry[2], A. Armento[2], M. Klausner[2] and P. Hayden[2]. [1]*AstraZeneca, Waltham, MA and* [2]*R&D, MatTek Corporation, Ashland, MA.*

The mechanisms of drug induced gastrointestinal (GI) toxicity are often poorly understood, in large part due to the lack of physiologically-relevant *in vitro* models. The inherent physiology differences between humans and preclinical animal models add another layer of complexity in interpreting GI toxicity studies. The objective of this work was to evaluate the utility of a new *in vitro* primary human cell based small intestinal (SMI) tissue model to predict drug induced GI toxicity. A blinded study was performed using 8 therapeutic compounds, including 5 for which dog and rat GLP toxicity studies were not predictive of human GI toxicity. These 5 compounds were selected from discontinued AstraZeneca clinical development programs and represent a diverse set of target classes and chemistries. As negative controls, the 3 other drugs included in this study were well tolerated in humans. To model GI toxicity, we examined cytotoxicity by MTT viability and LDH release assays and tissue barrier integrity using transepithelial electrical resistance (TEER) measurements, following two repeat exposures over 96 hours. The SMI model detected drug induced disruption of intestinal barrier function (TEER) in 5/5 problematic drugs at concentrations up to 30 fold below clinical exposure levels. Importantly, the SMI tissue showed no effect at concentrations up to 1,000 fold higher than the clinical exposure levels for the three negative controls. Overall, TEER measurements in the SMI tissue were a sensitive, predictive tool to assess clinically-relevant exposures of drugs that induced human GI toxicity that were incorrectly predicted in preclinical GLP toxicology studies. These results suggest that the *in vitro* system will serve as a useful tool for both investigational and traditional GI drug safety studies.

3070 Pre-Validation of *In Vitro - In Vivo* Assays for Vaginal Irritation

S. Ayehunie, T. Landry, P. Hayden and M. Klausner. *R&D, MatTek Corporation, Ashland, MA.*

A validated and predictive *in vitro* test system for assessing the vaginal irritation of test articles (TA) is needed to screen women's care products. The goal of the study was to validate the utility of the *in vitro* tissue model as an alternate vaginal irritation test by comparing head-to-head testing with the rabbit vaginal irritation (RVI) assay (ISO10993-10) for a

coded set of test articles (TA). A double-blinded study was conducted in which the investigator and the animal testing facility were provided with N=14 coded TA and assayed *in vitro* and *in vivo* following topical application at 2% dose of each TA and 5 repeat exposures over 6 days. Dose volumes were proportionally adjusted based on the surface area and N=5 rabbits and N=3 EpiVaginal tissues were used per TA. The RVI score was used to monitor *in vivo* irritation; MTT, TEER, and histological analysis were used as endpoints for the *in vitro* model. The results showed that four TA including two known irritants, benzalkonium chloride (BZK) and nonoxynol 9 (N9), were predicted as irritants by MTT viability and TEER (>50% reduction). BZK was identified as a mild/severe irritant in the RVI assay, but the effect of N9 in rabbits was highly variable - in three independent RVI studies, irritation was detected only in 25-30% of the animals. Furthermore, the other two TAs, copper sulfate and sodium dodecyl sulfate (SDS), that were determined as vaginal irritants *in vitro* were not identified as irritants in the RVI test. Cytokine analysis from culture supernatants showed that N9, SDS, and BZK induced a significant increase (>2 fold) in IL-1α/IL-1β release by EpiVaginal tissues. In contrast, only BZK showed a significant increase in IL-1α/IL-1β from rabbit vaginal lavages. In short, a combination of MTT, TEER, and IL-1α/IL-1β were found to be valuable markers of *in vitro* vaginal irritation. Tn vitro assay method performed equally well or better compared to the standard RVI assay and is anticipated to be a useful tool to assess vaginal toxicity of formulations, drugs, and medical devices.

PS 3071 Dose Response Studies Supporting *In Vitro* Based Safety Assessments for Chemicals Inducing Oxidative Stress

B. Huang[1], S. M. Ross[1], S. Rowley[1], A. Efremenko[1], S. Pendse[1], L. Pluta[1], P. Xue[2], J. Pi[3], P. L. Carmichael[4], A. White[4] and R. A. Clewell[1]. [1]*The Hamner Institutes for Health Sciences, Durham, NC;* [2]*Department of Environmental Health, Fudan University, Shanghai, China;* [3]*School of Public Health, China Medical University, Shenyang, China and* [4]*SEAC, Unilever, Colworth Science Park, United Kingdom.*

Oxidative stress is involved in the development of a variety of diseases including allergic and inflammatory skin diseases. To advance the transition to *in vitro* toxicity testing described in the National Academies of Sciences report, "Toxicity Testing in the 21st century (TT21C): A Vision and A Strategy", a series of assays to provide proof-of-concept *in vitro*- risk assessments have been developed for key readouts of Nrf2 oxidative stress toxicity pathway: oxidative homeostasis, Nrf2 transactivation, transcriptional regulation, protein and DNA oxidative damage (protein carbonylation, protein nitration, 8-hydroxy-2′-deoxyguanine), and cell fate (apoptosis, cytotoxicity). Dose-response data from these assays were collected from human immortal (HaCaT) and neonatal primary (HEKn) keratinocytes with curcumin and hydrogen peroxide exposure. Sorting biomarker response by no observed effect levels (NOELs) or benchmark dose (BMD) helped define the regions associated with sub-threshold, adaptive and adverse cellular responses. Primary keratinocytes were more susceptible to protein and DNA damage than the HaCaT cells. Transient protein damage (resolved within 24 hrs) occurred at much lower doses than pathway enrichment in the gene array studies. However, doses associated with transcriptomic pathway activation were similar to sustained protein and DNA damage and cytotoxicity. It appears that transcriptional activity response may not be the key protective mechanism against oxidative damage at low doses. We postulate that post-translational processes may be more important for resolution of protein damage at low doses. Further research is being conducted to evaluate the post-translational processes driving dose-response for cellular damage at low doses and to identify the tipping point for adversity in the oxidative stress pathway. This effort supports development of *in vitro*-based safety assessments for chemicals inducing oxidative stress.

PS 3072 Neutral Red Cytotoxicity Assay as a Screening Method to Aid in the Design of Compounds with Lower Acute Toxicities

D. L. Nabb[1], R. T. Mingoia[1], J. Yao[1], H. M. Peterson[1], L. K. Markell[1] and X. Han[2]. [1]*DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, DE and* [2]*International Flavors and Fragrances, Union Beach, NJ.*

The discovery stage in product research and development is a step spanning from product concept formation to significant financial commitment for development of that concept into the marketplace. A discovery toxicology program addresses the safety of a potential product and plays a significant role in the decision making process to determine advancement of the potential product to the next stage in development. Here we demonstrate how *in vitro* and *in vivo* screening approaches were used to aid in the design of compounds with lower acute toxicities. Initial screening tests with eight discovery analogs indicated minimal metabolism in rat hepatocytes, relatively high concentrations and long terminal elimination half-lives in plasma after a single oral exposure in rats, as well as acute toxicity in rats at 300 mg/kg. In order to develop a screening model for acute toxicity assessment, the Neutral Red Uptake (NRU) Cytotoxicity Assay was used to determine the potential for these test substances to cause cytotoxicity in Balb/3T3 mouse fibroblasts as a corollary to acute toxicity *in vivo*. The assay, based on the ability of viable cells to incorporate and bind the supravital dye neutral red, was performed in an eight-point dose-response format to determine the concentration that reduces neutral red uptake by 50% (IC50). A concentration-response curve was fitted to relate percent of solvent control viability to the logarithm of analog concentration and a linear regression model (Halle 1998, 2003) was applied to determine estimated oral LD50 values in mg/kg *in vivo*. The eight discovery compounds were rank ordered based on IC50 values, varying one logarithmic unit in scale, and showed a correlation between the analog rank order observed in the *in vivo* tests. These results demonstrate that the neutral red uptake assay may be a useful tool for rank ordering discovery compounds for acute toxicity potential *in vivo* as well as providing information to the chemists to guide the design of new analogs.

PS 3073 Early Gene Expression Changes During Mouse Embryonic Stem Cell Differentiation and Effects of Cadmium Sulfate

X. Chen[2], T. Han[2], E. Fisher[1], W. Harrouk[1], M. Tassinari[1], G. Merry[2], D. Sloper[2], J. Fuscoe[2], D. Hansen[2] and A. Inselman[2]. [1]*US FDA/CDER, Silver Spring, MD and* [2]*US FDA/NCTR, Jefferson, AR.*

The mouse embryonic stem cell test (mEST) is an *in vitro* stem cell based developmental toxicity test. The original mEST protocol used beating cardiomyocytes as the differentiation endpoint, but recently osteoblasts have been included as an additional endpoint. Both osteoblasts and cardiomyocytes correctly classified the skeletal teratogen cadmium sulfate (CS) as strongly embryotoxic. In the current study, early differentiation of mouse embryonic stem cells and embryotoxicity of CS were examined using transcriptomics. D3 mouse embryonic stem cells were exposed to vehicle or CS for 24, 48 or 72 hours and whole genome transcriptional profiles were determined. The results indicate a tract of differentiation that could be identified as culture time progressed. The differentially expressed genes increased over time in the unexposed culture, with the most significant changes occurring between 24 to 48 hours. A total of 1189 genes (false discovery rate < 0.05, fold change > 1.5) were differentially expressed at all 3 time points in the unexposed culture; biological processes that were associated with these genes included embryonic development and, specifically, skeletal system development. CS inhibited differentiation at all three time points. A total of 407 genes were identified as commonly responding to CS exposure. Functional pathway analysis of the 407 CS responsive genes indicated that in addition to the expected effect on genes involved in skeletal development, other toxicity pathways , including those related to nephrotoxicity, reproductive toxicity, and other types of developmental toxicity were also affected by CS. The results showed that transcriptional profiles are a sensitive indicator of differentiation. Transcriptomics could improve the predictivity e of mEST by identifying specific developmental toxicity pathways affected by chemical exposures.

PS 3074 *In Vitro* Developmental Neurotoxicity Testing Models Using HiPSC-Derived Neural Stem Cells

H. Yi, Y.-I. Park, J.-Y. Song and H.-G. Kang. *Animal and Plant Quarantine Agency, Anyangsi, Korea, Republic of.* Sponsor: H.-G. Kang.

Developing brain is particularly sensitive to chemical disturbances such as methyl mercury (MeHg). Because of the high cost of maintaining laboratory animals and animal welfare issue, standardized and predictive *in vitro* developmental neurotoxicity test system is critically required. HiPSC-derived neural precursor cells (iNPCs) can be used as a model for early brain development. We evaluated several *in vitro* test methods using iNPCs to predict adverse effects of low dose of MeHg on neural development. HiPSC-derived neural precursor cells (hNSC) purchased from Gibco (Carlsbad, CA) were maintained in specific media for neural stem cell maintenance (KnockOut DMEM/F12, StemPro® Neural Supplement with 20ng/mL of EGF and β-FGF). Differentiation of hNSC was induced to twenty-days in N2B27 media without EGF and bFGF. Well-known developmental neurotoxicant, MeHg were treated in 100, 200 and 400 nM. As quantitative evaluation methods for functional neurons, neurite outgrowth, fluorescence intensity of TUJ1 and membrane potential of neurons were measured by ArrayScan VTI HCS reader (Thermo Scientific)

and FlexStation 3 Benchtop Multi-Mode Microplate Reader. After 20 days of differentiation, hNSCs were turned into neurons with branching axons (neurites). These neurites predominantly expressed TUJ1, the neuron-specific proteins. Treatment of low dose of MeHg, 100, 200 and 400 nM during 20 days of differentiation did not reduced cell viability significantly. But the expression of TUJ1 was significantly reduced by 400 nM of MeHg. In high-contents screening (HCS) methods, we identified the toxic effects including alteration of mitochondrial membrane potential, accumulation of intra-cellular calcium by low dose of MeHg. In neurite outgrowth analysis, neurite length, neurite length with branches, neurite intensity, branch point count, branch point distance from cell body and cross point count showed a tendency to decrease by low dose of MeHg. Ion channel assay also showed that low dose of MeHg has the effect to decrease ion channel activity. In this study, we successfully applied advanced *in vitro* test methods as a new strategies to assess developmental neurotoxicity (DNT) of low dose of MeHg. These *in vitro* DNT test methods are applicable to high-throughput screening and provide a simple, predictive stem cell-based method for DNT testing.

3075 Abstract Withdrawn by Author.

3076 P-450 Activity in Human Pluripotent Stem Cell Derived Astrocytes Is Protective of Neuron Neurite Outgrowth Inhibition When Exposed to Chlorpyrifos

X. Wu[2,3], A. Majumder[1] and S. Stice[1,2,3]. [1]*ArunA Biomedical, Inc, Athens, GA;* [2]*Animal and Dairy Science, Regenerative Bioscience Center, Athens, GA and* [3]*Interdisciplinary Toxicology Program, University of Georgia, Athens, GA.*

Human neural progenitor (hNP) cells are capable of directed independent differentiation into astrocytes, oligodendrocytes and neurons and offer a potential cell source for DNT assays. These NP cell derived astrocyte and neuron can then be co cultured at ratios to ideally mimic brain cell composition and interaction. In our recently research, NP cell derived astrocytes have an integral role in the maintenance of early neuron differentiation and neurite extension, and are also associated with neuroprotection when they are exposed to toxic substances. Chlorpyrifos is organophosphate insecticide and affects the nervous system by inhibiting the breakdown of acetylcholine (ACh), a neurotransmitter. Cytochrome P450 enzymes (CYP) metabolizes chlorpyrifos to chlorpyrifos-oxon and chlorpyrifos-oxon is metabolized primarily to 3, 5, 6-trichloropyridinol (TCP) in addition to diethylphosphate and diethylthiophosphate. In the CNS, the levels of various cytochromes P450 enzymes are expressed at relatively high level in astroglial cells and may play a critical role in the biotransformation of endogenous or exogenous compounds. Our objective was to establish neuron alone and human astrocyte/neuron co-culture systems to identify the interactive role of astrocytes and neurons in chlorpyrifos induced neuron toxicity. In neuronal cell alone cultures chlorpyrifos inhibited neurite length, neurite number and branch point number per neuron in a dose dependent manner during a 48h exposure starting at 10 µM dose. However, astrocytes proved neuronal cell protective to chlorpyrifos at these same concentrations, up to 30µM chlorpyrifos. When CYP inhibitor SKF525A was added to these co cultures it negated the protective effect of astrocyte, reducing branch point number in the chlorpyrifos (10µM) plus SKF525A 5µM and 10µM treatment. We for the first time established in a scalable and defined cell number NP cell to astrocyte (10,000 cells/well) and neuron (10,000 cells/well) co-culture model and potentially identify the role of P450 in astrocyte neuroprotection.

3077 Rotenone-Induced Mitochondrial and Nuclear DNA Damage Is Reversed During PC12 Cell Differentiation

R. Joglekar[2], I. T. Ryde[2], S. K. Murphy[2] and J. N. Meyer[2]. [1]*Department of Obstetrics and Gynecology, Duke University, Durham, NC and* [2]*Nicholas School of the Environment, Duke University, Durham, NC.*

Most mechanistic studies of DNA damage have been carried out in differentiated cells and fully developed organisms. The effects may be different for a developing embryo. For example, exposure to xenobiotics that induce reactive oxygen species (ROS) can result in oxidative DNA damage, which could impact the genomic stability of differentiating cells. The ways in which xenobiotic-induced ROS affect development is not well characterized. Due to the expense and difficulty of mammalian studies compounded with the multitude of ROS-generating xenobiotics, it is essential to develop effective high-throughput *in vitro* screening assays to examine the potential developmental effects of xenobiotic-induced oxidative DNA damage. Here, we used PC12 cells, derived from rat adrenal medulla tumors, as a model to study the effects of xenobiotic-induced oxidative DNA damage on a differentiating cell. PC12 cells are pluripotent like primitive progenitors, and can differentiate into sympathetic neurons if exposed to nerve growth factor (NGF). Thus, they serve as a powerful model to examine exposure effects during a simulated developmental period consisting of cellular reprogramming. We exposed cells to the pesticide rotenone, a mitochondrial complex I inhibitor and generator of ROS. Cells were exposed to 0, 0.5, 1, and 5uM rotenone and 0.1% NGF for 6 days, and nuclear (nDNA) and mitochondrial DNA (mtDNA) damage were quantified using Q-PCR. We found a time-dependent reversal of both nDNA and mtDNA damage after 3 days of exposure and a reappearance of damage at 6 days, suggesting a critical period in differentiation during which damage is better repaired and after which damage accumulates. nDNA and mtDNA damage reached its peak of 0.6 lesions/10kb on day 2, dropped below the limit of detection on day 3, and reaccumulated to 0.2 lesions/10kb by day 6. To confirm these results in a human model, we will expose human neural progenitor stem cells to rotenone and other priority toxicants, and measure nDNA and mtDNA damage and mitochondrial function as endpoints for genomic instability and mitochondrial dysfunction. We are also currently conducting epigenetic assays in the PC12 model to examine the potential changes in CpG methylation patterns as a result of xenobiotic-induced ROS.

3078 Mechanistic Toxicity Assessment of Hexahydroisohumulone in Canine Hepatocytes, Renal Proximal Tubules, and Enterocyte-Like Cells

K. Choi[2], J. Koci[2], M. T. Ortega[2], B. Jeffery[1], J. E. Riviere[2] and N. A. Monteiro-Riviere[2]. [1]*Mars Global Food Safety Center, Yanqi Economic Development Zone, Huairou, Beijing, China and* [2]*Anatomy and Physiology, Kansas State University, Manhattan, KS.*

In vitro test methods are used primarily for rapid screening of chemicals based on mechanistic understanding of toxicity for the prediction of hazards and potential risks. Here, we investigated organ-specific oxidative stress and molecular mechanisms of toxicity using pathway-focused DNA array of the hop ingredient hexahydroisohumulone (HEX) with 4 different canine cell systems: hepatocytes, canine proximal tubule cells (CPTC), bone marrow-derived stem cells (BMSC) and enterocyte-like cells (ELC). Free radical species were extensively produced in HEX-treated hepatocytes and to a lesser extent in CPTC, BMSC and ELC. Transcriptional profiles showed 30.5% (113 genes) of the total genes (370 genes) differentially expressed in hepatocytes followed by CPTC (21.6%, 80 genes), ELC (4.8%, 18 genes) and BMSC (1.0%, 4 genes). HEX predominantly affected DNA damage/repair pathway in hepatocytes and CPTC, but for ELC the ER stress/unfolded protein response dominated. HEX also suppressed antioxidant and phases I and II drug metabolizing enzymes at the transcriptional levels. The cyclooxygenase-2 (COX-2) and DNA damage-inducible transcript 3 (DDIT3) were the most abundant genes in HEX-treated hepatocytes and CPTC. The COX-2 and DDIT3 both networked separately and complementary between various pathways resulting in HEX-mediated adverse effect on apoptosis, oxidative stress, ER stress/unfolded protein response, β-oxidation and mitochondrial metabolism. This work contributes to the comprehensive analysis of the molecular effects of HEX and the cellular response to oxidative stress in four cell types representing different organ-specific functions and provides insight into the genes that may be of significant importance in the safety assessment of HEX in dogs. (Supported by Mars Global Food Safety Center, Inc.)

3079 Comparative *In Vitro* Cytotoxicity of 20 Potential Food Ingredients in Canine Liver, Kidney, Bone Marrow-Derived Mesenchymal Stem Cells and Enterocyte-Like Cells

N. A. Monteiro-Riviere[2], M. T. Ortega[2], K. Choi[2], J. Koci[2], Z. Lin[2], B. Jeffery[1] and J. E. Riviere[2]. [1]*Mars Global Food Safety Center, Yanqi Economic Development Zone, Beijing, China and* [2]*Anatomy and Physiology, Kansas State University, Manhattan, KS.*

To begin development of a mechanistically-relevant humane alternative platform for safety assessment of dog food ingredients, comparative *in vitro* cytotoxicity of 20 ingredients was assessed in four canine cell types relevant for toxicity assessments. Previously, we described the toxicity of 13 compounds [clove leaf oil, eugenol, guanosine monophosphate (GMP), GMP plus inosine monophosphate, sorbose, ginger root extract, cinnamon bark oil, cinnamaldehyde, thyme oil, thymol, lemon grass oil, xylitol, and citric acid] using *in vitro* primary canine cell culture

models for liver, kidney, bone marrow-derived mesenchymal stem cells (BMSC) and enterocyte-like cells (ELC). In this report, dose-response cytotoxicity studies and LC50 using alamar blue assays are reported for 7 additional compounds: denatonium benzoate, eucalyptol, hexahydroisohumulone, tetrahydroisohumulone, green tea catechin extract, epigallocatechin gallate, and sodium copper chlorophyllin. Data across 20 compounds were compared between different cell types and responses were not parallel, precluding the use of a single cell line for *in vitro* ingredient hazard assessment. Hepatocytes were most resistant to all compounds, consistent with their xenobiotic detoxification functions. BMSC and ELC showed an increase in sensitivity to the essential oils eucalyptol, eugenol, and thymol compared to renal cells and hepatocytes. These studies provide a baseline of acute cytotoxicity of four canine cell types to 20 different food components that begin to illustrate how such an *in vitro* panel could be used for hazard assessment. (Supported by Mars, Inc.)

PS 3080 A Synthetic Alternative to Natural Extracellular Matrix Vascular Network Formation Assays

W. T. Daly, E. H. Nguyen, D. Belair, N. N. Le and W. L. Murphy. *Department of Orthopaedics and Rehabilitation, UW-Madison, Madison, WI.*

Introduction: Matrigel has remained the most commonly used substrate for the screening of pro- and anti-angiogenic compounds. This xenogenic matrix is composed of over 1800 individual proteins which varies from lot-to-lot and batch-to-batch with poor handling properties. In this study we hypothesized that simple, well-defined synthetic hydrogels could provide alternative substrates to Matrigel for use in vascular screening systems. Here, we used an enhanced throughput hydrogel array to determine chemically defined synthetic hydrogel formulations that permit formation of vascular networks similar to those on Matrigel. After establishing a suitable substrate, we subsequently tested its efficacy by screening a panel of known and unknown vascular disrupting compounds. Methods: A library of over 250 different synthetic hydrogel formulations was screened to identify vascular network forming conditions with both primary human umbilical vein endothelial cells and induced pluripotent stem cell derived endothelial cells. Networks were evaluated based on their stability, structure and their response to a panel of known vascular and molecular inhibitors in comparison to Matrigel. After identification of a suitable substrate, the newly developed vascular screening platform was used to screen 53 putative vascular screening compounds from the EPA Toxcast library. Results & Discussion: The identified synthetic matrix had several advantages over Matrigel including: control over all properties of the matrix including local growth factor sequestration and cell-mediated degradation, increased repeatability and sensitivity as well as translatability to automated liquid handling and high-throughput screening systems. In the testing of known inhibitors the synthetic matrix showed increased sensitivity and reproducibility compared to the Matrigel substrate. An additional feature, was the ability to locally sequester growth factors within the synthetic matrix. Interestingly, the inclusion of this feature demonstrated the ability to nullify the effects of a number of known anti-angiogenic compounds and could be a potential mechanism that would cause these compounds to be ineffective *in vivo*. Conclusion: A modular synthetic hydrogel formulation was identified for screening of potential pro- and anti-angiogenic compounds and demonstrated superior performance to Matrigel.

PS 3081 Optimizing Clustering Strategies for Chemical Categories: Toxicological Verification of Derived Clusters

A. Bitsch[2], M. Batke[2], U. Gundert-Remy[1], M. Gütlein[3], S. Kramer[3], F. Partosch[1] and M. Seeland[3]. [1]*Charité Universitätsmedizin, Berlin, Germany;* [2]*Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany and* [3]*Johannes Gutenberg University of Mainz, Mainz, Germany*. Sponsor: C. Dasenbrock.

According to OECD a chemical category is a group of chemicals whose physicochemical and human health and/or ecotoxicological properties are likely to be similar or follow a regular pattern. We established an approach by which toxicological and structural properties likewise contributed to the building of chemical categories for (sub)chronic toxicity. Two databases on repeated-dose toxicity (RepDose and the "ELINCS" data base) served as basis for a multi-label clustering using predictive clustering trees (PCT). The clustering was performed based on chemical structure and physicochemical properties. The resulting clusters were evaluated in a two-dimensional approach including their toxicological profile to optimize the clustering strategy. Here, we analyse clusters for specific (sub)structures and/or common mechanisms of toxicity to establish the plausibility and quality of clustering results. For special (sub) structures such as nitro compounds or glycols and alcohols the decision tree for cluster separation was followed and the result was evaluated to be meaningful with relation to structural properties, toxicological profiles and/or key events and i.e. for nitro groups in addition to toxic potency. In addition, clusters were evaluated based on the toxicological profile, on special target organ or addressing expected effects due to known mechanisms of toxicity. In our dataset were i.e. 63 substances affecting the thyroid; in a mechanistic approach the dataset was searched for structures, which stimulate or inhibit thyroidal function. As key events the regulation of iodine up-take, thyroxine synthesis and the inhibition of thyroid peroxidase were identified in the clusters. Thus, several pathways are involved and the distribution of the compounds in different clusters is toxicologically justified. Clusters showing toxicological effects in spleen were very limited in size and it seemed that the endpoint is not covered well by the clustering. However, there are pathophysiological connections between the organs spleen and bone marrow and effects like anaemia and haematopoiesis; an evaluation based on these correlations showed very convincing results. All in all, the clustering approach showed very promising results.

PS 3082 Development of a Model of Dermal Inflammation and Irritation (Urticaria) in the Miniature Swine

A. Stricker-Krongrad, M. Zhong, J. Liu and G. Bouchard. *Sinclair Research Center, Auxvasse, MO.*

Urticaria is dermal edema that results from vascular dilation and leakage of fluid into the skin in response to molecules released from mast cells. Our objective was to develop a reproducible dermal urticaria model in miniature swine. Three Hanford minipig (3 to 18 months old) were used in the study. The animals were pricked on their back skins with a skin test device (Lincoln Diagnostics Inc, Decatur, IN) that were loaded with vehicle or histamine. The irritation and wheal and flare responses of the dermis were evaluated with Draize scoring and with wheal size measurement. The reactions of the skin were assessed at 10, 20 or 25, and 45 minutes after test formulation application. Histamine dose-dependently induced skin irritation at both 10 and 20 minutes after treatment. The most prominent erythema and edema (Draize score) responses were observed at 10 minutes after treatment, which were slightly diminished at 20 minutes after treatment. Histamine also caused skin wheal that ranged between 4-7 mm in diameter. When urticaria was induced with 25 and 50 mg/mL histamine, the topical administration of Allegra as well as Cortaid inhibited both the dermal irritation and the wheal and flare responses. The inhibitory effects of Allegra and Cortaid were observed at 25 and 45 minutes post dose. In conclusion, a histamine-induced urticaria model has been established in the Hanford miniature swine and can be used for the testing of topical treatments for dermal irritation and inflammation.

PS 3083 Comparative Safety and Efficacy Assessment of Single and Concurrent Topical Therapeutics in an IMQ-Induced Murine Model of Psoriasis

V. Naageshwaran, T. Jackson, I. Ruiz and A. Alavi. *Absorption Systems, San Diego, CA.* Sponsor: C. Davenport.

Purpose: Psoriasis vulgaris is a human autoimmune disease that manifests as an inflammatory skin condition characterized by focal and coalescing cutaneous plaques with consistent scaling and variable erythema. The disease etiology and pathogenesis are not fully understood but the underlying mechanisms involved include excessive proliferation of the keratinocytes as well as an immune mediated response involving the activation of T cells. A preclinical model which recapitulates the phenotype of the clinical disease could be utilized to determine the efficacy and safety of novel and generic topical therapeutics that may be used concurrently. Methods: Daily topical treatment of male and female CD-1 mice with imiquimod (IMQ) was performed for 7 days resulting in the development of psoriatic lesions as well as hyperplasia of the epidermis (measured by dorsal skin and ear thickness). Clinical observations, Draize scoring and body weight measurements were recorded upon disease induction and following treatment by various topical therapeutics (calcineurin inhibitor tacrolimus alone and in combination with corticosteroid clobetasol) for 24 hours, 48 hours, and at various time points (3, 5, 7) out to 10 days. Histological analysis (H&E staining) of the skin of the back and ear was performed from treated and vehicle (saline) control animals to evaluate the size of the lesions, extent of hyperkeratosis and inflammatory biomarkers in the dermis. Results: IMQ treatment resulted in onset and development of phenotypic symptoms of psoriasis including inflammation and increased thickness of the dorsal skin and ears. Topical application of the different therapeutic agents singly and concurrently showed efficacy with the greatest differences observed in the animals treated with the corticosteroid clobetasol. Conclusions: The

IMQ-induced mouse psoriasis model presents hyperproliferation and differentiation of epidermal cells and the presence of inflammatory cells. In the current study the model was utilized to evaluate calcineurin inhibitor tacrolimus alone and in combination with corticosteroid clobetasol. This provides the framework to establish a model that can be used to show the safety and efficacy of concurrently used topical medications that may have varied mechanisms of action.

3084 Prevalence and Incidence of Cataracts in a Population of Yucatan Miniswines After Induction of Type I Diabetes: A Model for Preventative or Therapeutic Cataract Therapies

A. Stricker-Krongrad[2], C. Hanks[2], A. Ingerson[2], M. Freeman[2], S. Schlink[2], L. Delaney[2], J. Liu[2], A. Tellez Cruz[1], J. Wicks[1], S. Rousselle[1] and G. Bouchard[2]. *[1]Alizee Pathology, Thurmont, MD and [2]Sinclair Research Center, Auxvasse, MO.*

Cataracts as a consequence of chronic diabetes is considered a leading cause of legal blindness in humans in the United States and is also observed frequently in aged diabetic populations (>65%). We assessed post-induction (PI) onset of clinical ocular cataract(s) in a colony of over 266 castrated, male, diabetic, Yucatan miniature swine. Over the course of a 6 month period, the prevalence was 30% (80 positive animals out of 266 animals). The most recent incidence (past 2.5 months) was 20.4% (38 positive animals with 60 affected eyes from pool of 186 previously negative animals). Eighteen animals had bilateral and 20 animals had unilateral cataracts (OD: 31; OS: 29). Cataract onset ranged from 2 to 19 months PI with an average of 11 months PI. Cataracts were detected earlier in animals when euglycaemia was intentionally less controlled, which supports the current predominant theory of glycation-induced cataract development. Interestingly, swine unlike human are not capable of glycating their hemoglobin due to the lack of penetration of glucose into the red cells. Miniswine with cataracts appear to function acceptably well despite the assumed visual handicap by relying on other senses. In conclusion, diabetic Yucatan miniature swine commonly manifest with cataracts on average at 11 months post-induction. Insulin regimen and glucose control are strong factors in the prevalence and incidence of cataracts in diabetic miniswines. Our data also suggests that the glycation of swine lenses readily occurs due to the high incidence of cataracts in diabetic animals with non-optimal glucose control. In addition the diabetic miniswine would provide a good model for preventative or therapeutic cataract therapies.

3085 Therapeutic Effect of Acamprosate Calcium in a Rat Model of Tardive Dyskinesia

C. S. Godin[4], S. Bachus[2], G. Gesswein[4], N. Moore[1] and W. Kerns[3]. *[1]Brains-Online, South San Francisco, CA; [2]George Mason University, Fairfax, VA; [3]Synchroneuron, Inc., Waltham, MA and [4]Toxicology Services, Smithers Avanza, Gaithersburg, MD.*

Acamprosate modulates glutamatergic and GABAergic activity in the brain, and is approved for the treatment of alcoholism. The therapeutic effect of acamprosate calcium (ACP) was evaluated in male Sprague-Dawley rats with haloperidol-induced vacuous chewing movements (VCMs) over a period of approximately 23 weeks. Four groups of rats were evaluated: vehicle control, haloperidol (H)-28.5 mg/kg IM every three weeks, H plus oral ACP at 330 mg/kg/day, and H plus oral ACP at 600 mg/kg/day ACP. Evaluations included morbidity/mortality, physical examinations, cage side observations, body weights, food consumption, water consumption, pharmacokinetics, and VCM counts. VCM data were collected under quiet conditions once weekly during the study in a single-blinded manner by an experienced investigator. Acamprosate exposure was proportional to dose. Haloperidol with or without ACP markedly reduced the incidence of alopecia and abrasions reflecting the binding of haloperidol to dopamine receptors in the brain. Haloperidol with or without ACP also resulted in a decrease in body weight gain, food consumption, and a gradual reduction in water consumption when compared to the concurrent controls. Haloperidol induced VCMs within one week of the first dose, and ACP exposure decreased the haloperidol-induced VCMs starting after 15 weeks of dosing; this effect is consistent with acute VCMs being EPS-related (Extrapyramidal Symptom) and late-stage VCMs being TD-related. In conclusion, under the conditions of the study Acamprosate calcium was both safe and therapeutically effective in this chronic model of orofacial dyskinesia in rats.

3086 Efficacy and Metabolic Impacts of Risperidone in an Insulin-Resistant Mouse Model of Autistic Behaviors

V. R. Garbarino, M. T. Edwards, R. Lozano, C. M. Smolik, W. Q. Zhang, L. C. Daws and G. G. Gould. *Physiology, UTHSCSA, San Antonio, TX.* Sponsor: G. Gould.

Autism Spectrum Disorders (ASD) are persistent developmental disorders classified by the symptoms of social impairments and restrictive repetitive behaviors. Approximately 1 in 68 children have autism, showing up 4 times more often in males. There is presently no comprehensive treatment for all autism symptoms, but patients are most commonly given risperidone, an atypical antipsychotic to control impulsivity and aggression. Risperidone has tranquilizing properties, acting as an antagonist at serotonin and dopamine receptors. Risperidone's long-term use comes with serious side effects including increased appetite, obesity, and early onset of type II diabetes. We hypothesize that because chronic risperidone treatment can accelerate onset of metabolic syndrome, this drug might ironically blunt its own effectiveness by inducing elevated insulin levels that modify the expression and functions of dopamine transporters and receptors. We sought to identify a mouse model in which to test this. Black-and-tan Brachyury Tuffed mice, crossed with C57BL/6 mice yield F1 hybrid offspring that are socially impaired and glucose deficient. F1 mice have increased abdominal adiposity, and elevated insulin levels relative to either parent strain by 10 weeks of age. Their social and repetitive behavior was not improved by risperidone administered acutely. F1 mice were more impulsive than either parent strain in a social dominance test. [3H] WIN 35,428 binding to dopamine transporters was similar among strains, but dopamine uptake was significantly increased in BTBR mice. Dopamine transmission may be suppressed in the F1 mice, which may alter their responsiveness to risperidone. As evidence of this, marble burying in F1 mice was unaffected by an acute risperidone treatment, while the same dose of risperidone reduced this in C57BL/6 mice. The BTBRxBL6 F1 mouse is a useful physiological model for investigation of long-term drug treatment effects on the social brain and on glucose metabolism as seen in ASD.

3087 The Cynomolgus Macaque Model of Pneumonic Tularemia

L. Lanning[3], P. Sanz[3], L. Wolfraim[3], C. Houchens[2], K. Omland[1], M. Williams[3], J. Hewitt[3] and T. Guina[3]. *[1]Mergus Analytics, Jericho, VT; [2]CBRN, BARDA, Washington, DC and [3]OBRRTR, NIH/NIAID/DMID, Bethesda, MD; [4]ORA, NIH/NIAID/DMID, Bethesda, MD.*

The National Institute of Allergy and Infectious Diseases (NIAID) and the Biomedical Advanced Research and Development Authority (BARDA) formed a Working Group for qualification of the cynomolgus macaque (*Macaca fascicularis*) model of pneumonic tularemia as a Drug Development Tool with the Food and Drug Administration (FDA). Qualification of an animal model through FDA's Animal Model Qualification Program (AMQP) is limited to those proposed for use in product approval under the Animal Rule. This is a regulatory mechanism for approval based on evaluation of the efficacy of new-generation therapeutics and vaccines in suitable animal models [21 CFR 314.600 for drugs; 21 CFR 601 Subpart H for biological products]. The adequacy of an animal model for qualification is based on data from natural history studies and from the human disease or condition showing that the specific animal species given a specific challenge agent by a specific route, produces a disease that corresponds to the human disease or condition of interest in multiple important aspects. We intend to qualify the natural history model of pneumonic tularemia in the cynomolgus macaque based on results of the LD50 and natural history studies that have been completed at Battelle Biomedical Research Center (BBRC), Lovelace Respiratory Research Institute (LRRI) and United States Army Medical Research Institute for Infectious Diseases (USAMRIID) in 2007-2015. This presentation includes the general characteristics of pneumonic tularemia in cynomolgus macaques exposed to the target dose of 1,000 cfu aerosolized *F. tularensis* Schu S4 based on the results of completed LD50 and natural history studies. Mortality, average time to death, average time to fever onset, average interval between fever and death, initial level of bacteremia post-challenge, timing of bacteremia onset, clinical signs, necropsy findings and histopathology are presented. These characteristics form the basis for the proposed cynomolgus macaque model parameters that are currently under discussion with the FDA in support of the model qualification. Data collected to date support the conclusion that symptoms and signs of pneumonic tularemia in cynomolgus macaques exposed to a target dose of 1,000 cfu (range 300 to 3000 cfu) aerosolized *F. tularensis* Schu S4 and human pneumonic tularemia cases are highly similar.

3088 Comparative Sensitivity and Reliability of a Multi-Analytes Platform and a Multiplex Flow Cytometry in the Quantitation of Circulating Cytokines Level in a Mouse Model of Edotoxemia

A. Stricker-Krongrad, J. Liu, G. Bouchard and M. Zhong. *Sinclair Research Center, Auxvasse, MO.*

A number of cytokines, such as interleukin 2 (IL-2), interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), IL-4, IL-6, IL-10 and IL-17A, are elevated in response to inflammation and are also key regulators of immune responses. Hence, measuring the expression profiles of these cytokines is important in monitoring the polarization of the immune response and therefore the results should be independent of the quantitation methods. To evaluate this effect, the 7 circulating Th1/Th2/Th17 cytokines were quantified in plasma of lipopolysaccharide (LPS +/- dexamethasone versus vehicle) treated mice with two different multiplex platforms (Myriad-RBM and BD Biosciences). Female C57BL6 mice (6-9 months old) were treated with vehicle (0.5% methyl cellulose in water) or dexamethasone (5 mg/kg), followed by LPS (0.2 mg/kg) via intravenous injection (IV). At 0.5, 1, 2, 4, and 6 hours after LPS challenge, blood samples were analyzed with the Rodent MAP V3.0 Antigens assay, with the Mouse Cytokine Panels A & B assay (Myriad-RBM; Luminex platform) and compared to the Mouse Th1/Th2/Th17 cytokines (Becton-Dickinson: BD CBA) assay on a BD Accuri C6 flow cytometer platform. TNF-α, IL-2, IL-4, IFN-γ, IL-6 and IL-10 were shown to be the early phase responders, and IL-17A was shown to be a sustained responder, which was up-regulated through LPS treatment. Reproducible circulating TNF-α and IL-6 levels were obtained for plasma samples of the LPS treated mice with the assays from both Myriad-RBM and BD Biosciences. The BD CBA cytokine assay was not as sensitive as the Myriad-RBM assays in detecting and quantitating circulating IL-2 and IL-4 and IL-17A levels in the LPS treated mice, but was more sensitive and reliable in measuring circulating IFN-γ levels. Reliable circulating IL-4 measurements were not achieved by either assay.

3089 The Effects of a High Fat (HF) Diet on Male Diversity Outbred (DO) Mice

M. J. DeVito[2], S. J. Borghoff[3], L. Zorrilla[1], K. R. Shockley[4], G. Kissling[4] and G. Travlos[2]. [1]*Bayer Crop Science, Research Triangle Park, NC;* [2]*Division of the National Toxicology Program, Research Triangle Park, NC;* [3]*ToxStrategies, Inc., Cary, NC and* [4]*NIEHS, Biostatistics and Computational Biology Branch, Research Triangle Park, NC.*

The effects of a HF diet on male DO mice were evaluated after 14 weeks on the diet. Mice were fed ad libitum, except during designated fasting periods, either a control diet (10 kcal % fat; D12450J) or a HF diet (60 kcal %; D12492) from the start of the study (n=75 mice per diet group). Serum insulin and leptin were determined one week prior to the start of the study and at study termination; week 14. Blood glucose was measured one week prior to the start of the study and during weeks 5, 9 and 14 using a Blood Glucose Monitoring System Meter. At termination, body, liver, brain, kidney, testes, and epididymis weights were recorded. Epididymal sperm motility and concentration parameters were measured by computer-assisted sperm analysis (Hamilton-Thorne,) per National Toxicology Program (NTP) specifications. Upon arrival, mice weighed approximately 23.6 ± 2.9 g. After 14 weeks mice on the HF diet weighed approximately 30% more than animals on the control diet (43.8 g vs 33.2 g, respectively). Mice on the HF diet gained more than twice as much weight as the controls (20.2 g vs. 9.7g, respectively). Approximately ¼ of the mice on the HF diet had mean blood glucose levels equivalent to the controls and approximately ¼ of the mice had mean blood glucose levels twice the control level. The most striking observation was the 70-fold range in sperm counts in the DO mice. In comparison, at the NTP, sperm count varies no more than 2-fold within a study in the B6C3F1 mice. Approximately ¼ of the mice on the HF diet did not differ from controls in body weight or clinical chemistry. Compared to the B6C3F1 mice typically used at the NTP, the coefficient of variation for organ and body weights is approximately 3-6 times higher in the DO mice. Using power calculations (80% power at $p < 0.05$), studies of most traits would require 25-34 mice in order for DO mice to be equally powered as a typical subchronic study using the B6C3F1 mouse. However, sperm count studies would require 150 mice/group to have the same power as 10 mice/group of the B6C3F1 strain due to increased variability in the DO. This study demonstrates that for some endpoints the DO mice vary greatly, while other endpoints the variance is slightly higher than the B6C3F1 mouse.

3090 A 52-Week Study of the Long-Term High Sugar High Fat Diet Effects on Cynomolgus Monkeys

N. Lalayeva[2], S. Nechev[2], T. Beck[2], T. Rogers[2], S. Glaza[2], P. Franklin[2], H. Tsusaki[2], K. Fukuzaki[2] and R. Nagata[1]. [1]*Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan and* [2]*SNBL USA, Ltd., Everett, WA.*

Sixty naïve male cynomolgus monkeys were divided into a control group and an intervention group and were offered either a standard primate lab animal diet or a commercially available high sugar high fat (HSHF) diet (LabDiet 5L0P) twice daily for 10 months. The HSHF diet was well tolerated and resulted in expected increases in somatometric parameters, setting the stage for obesity-associated insulin resistance, metabolic syndrome or T2D. While the control group mean body weights remained consistent with baseline values, those of the intervention group increased steadily for 20 weeks. After 10 months, the control group mean body weight was 7.5 kg, while that of the intervention group was 10.4 kg. Animals in the intervention group developed predominantly central adiposity and dyslipidemia (abdominal circumference, BMI, trunk fat, serum triglycerides and total cholesterol increased 1.6-fold, 1.4-fold, 1.7-fold, 1.6-fold and 1.5-fold, respectively, relative to the control group). Mean fasting glucose in the intervention group remained consistent with baseline and concurrent control group values; however, the upper limit of normal (ULN) was consistently exceeded in individual animals from the intervention group as early as 15 weeks into the trial. Expected increases (glucose) and decreases (insulin) in concentrations and AUC were also apparent from the ivGTT test conducted during weeks 21/22. Based on the fasting glucose and ivGTT results, animals in the intervention group were divided into normal glucose tolerance-insulin sensitive (NGT-IS), normal glucose tolerance-insulin resistant (NGT-IR), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and overt diabetes. HbA1c was predictably increased in individual animals in the intervention group. Expected changes were noted in adiponectin (decrease) and leptin (increase). This study demonstrated that consumption of HSHF diet in cynomolgus monkeys results in components of the metabolic syndrome or overt T2D. Based on insulin and glucose concentration (fasting and ivGTT), animals can be segregated into different categories for a better understanding of the pathogenesis and treatment options of metabolic disorders in humans.

3091 Comparison of Methionine and Choline Deficient (MCD) Diet-Induced Non-Alcoholic Steatohepatitis (NASH) with Methionine and Choline Sufficient (MCS) and Control Diet in Rats

V. Bhaskaran, D. M. Nelson, E. Janovitz, Y. Song, B. Lehman, D. Bounous, U. Hanumegowda and L. Lehman-Mckeeman. *Bristol-Myers Squibb, Princeton, NJ.* Sponsor: U. Hanumegowda.

In the present study, we characterize temporal changes in biochemical and liver histology in MCD diet-induced NASH and compare with MCS and control diets in Sprague Dawley rats. Male rats were fed either standard chow (Teklad 2018C), MCS (formulated diet with L-Methionine 3gm, Choline bitratrate 2gm) or MCD diet for 8 or 12 weeks. Groups of rats were necropsied at the end of weeks 1, 2, 4, 8 or 12. In MCD-fed rats there was a reduction in food consumption as early as week 1 correlating with a decrease in body weight gain throughout the study. Liver weights increased on an average 1.5 fold from week 2 through 12 which correlated with hepatocellular steatosis. Serum biochemical parameters were consistent with perturbed liver function and injury and included time dependent increases in AST, ALT, bilirubin and bile acids and decreases in triglycerides, cholesterol, HDL and LDL. Hepatocellular steatosis was seen by the end of week 1 and diffuse macro-vesicular steatosis by the end of week 2. Steatohepatitis, fibrosis, and cholangiocellular and hepatocellular hyperplasia were seen by the end of week 8, which progressed in severity by week 12. MCS diet did not affect food consumption and body weight when compared to regular chow. However liver weight increased 1.14 fold in MCS group by week 8 and serum TG increased whereas LDL decreased from week 1 onwards. Minimal to moderate micro and macrovesicular steatosis, centrilobular apoptosis and mild multifocal mononuclear inflammation were observed in the liver at week 8. These results demonstrate that MCD is a robust model for NASH and that a synthetic methionine and choline replete diet does not completely ameliorate the development of steatosis.

3092 Enhanced Susceptibility to Acetaminophen-Induced Liver Injury in C57Bl/6N Compared to C57Bl/6J Mice

L. Duan[1], B. L. Woolbright[1], K. Du[1], J. Weemhoff[1], M. Bourdi[2] and H. Jaeschke[1]. [1]*University of Kansas Medical Center, Kansas City, KS and* [2]*Molecular and Cellular Toxicology Section, Laboratory of Molecular Immunology, National Heart, Lung, National Institutes of Health, Bethesda, MD.*

Acetaminophen (APAP) overdose is a major cause of drug-induced liver failure. Overdose of APAP results in formation of protein adducts, mitochondrial dysfunction, release of endonucleases and massive hepatic cell death. Previous studies have indicated that there might be differences in susceptibility between different sub-strains of C57Bl/6mice, with animals of the C57Bl/6N substrain being more susceptible than its C57Bl/6J counterpart. This is particularly interesting in light of recent increases in the use of knockout mouse models generated in the C57Bl/6 background. This study was initiated to determine the mechanism behind the increased susceptibility of C57Bl/6N to APAP. Fasted C57Bl/6N (Charles River) and C57Bl/6J mice (Jackson Labs) were dosed with 200 mg/kg APAP and then sacrificed at different time points. C57Bl/6N mice had significantly more liver injury as measured by plasmas ALT activities and histology. Although, there was no difference in early glutathione depletion after APAP or P450 activity between the groups, significantly more APAP protein adducts were measured in C57Bl/6N mice. These animals also had significantly more mitochondrial translocation of bax and phospho-JNK, higher GSSG levels and more release of apoptosis inducing factor and smac protein from the mitochondrial intermembrane space. These findings suggest that the C57Bl/6N mice experienced an increased mitochondrial oxidant stress and more severe mitochondrial dysfunction. The increased mitochondrial dysfunction was confirmed in cultured hepatocytes *in vitro* as hepatocytes from C57Bl/6N mice had a significantly more precipitous drop in JC-1 fluorescence after APAP exposure; despite the fact that *in vitro* cell death was similar in the two sub-strains. In conclusion, mice of the C57Bl/6N substrain are more susceptible to APAP than C57Bl/6J mice, likely due to increased formation of APAP-protein adducts and a subsequent enhancement of the mitochondrial dysfunction.

3093 *In Vivo* Model for Predicting Organophosphate Toxicity using Humanized-Liver TK-NOG Mouse

H. Suemizu[2], M. Kuronuma[2], K. Kawai[2], N. Murayama[3], M. Nakamura[1] and H. Yamazaki[3]. [1]*Department of Pathology and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan;* [2]*Laboratory Animal Research Department, Central Institute for Experimental Animals, Kawasaki, Japan and* [3]*Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Tokyo, Japan;* [4]*Pathology Analysis Center, Central Institute for Experimental Animals, Kawasaki, Japan.* Sponsor: H. Tsutsumi.

The diagnostic procedure for acute organophosphorus pesticide (OP) toxicity in humans involves the definitive measurement of plasma butyrylcholinesterase (BuChE) activity. However, BuChE is not used for risk assessment in experimental animal models because its inhibition is not considered an adverse effect. In the humanized-liver (Hu-liver) TK-NOG mouse model, the liver is replaced with transplanted human hepatocytes and therefore, produces human hepatic proteins including drug metabolizing enzymes and secretory proteins. The plasma BuChE activity of Hu-liver TK-NOG mice was incomparably higher than that of non-humanized TK-NOG mice (258 ± 127 and 28 ± 10 IU/L, n = 309 and 36, respectively), but comparable to that in humans. In this study, we evaluated plasma BuChE activity in Hu-liver TK-NOG mice to determine if it could serve as a predictive marker for OP toxicity. Single oral administration of non-lethal doses of acephate and chlorpyrifos (300 and 10 mg/kg, respectively) to Hu-liver TK-NOG mice almost completely abolished the plasma BuChE activity at 7 and 0.5 h, respectively. A dose-dependent decrease in plasma BuChE activity was observed at the most effective doses of acephate and chlorpyrifos, which ranged from 1 to 300, and 0.1 to 3 mg/kg, respectively. The irreversible inhibition of plasma BuChE activity was recovered following the synthesis of new plasma proteins by the humanized-liver cells. Benchmark dose (BMD) analysis of the data from the BuChE inhibition induced by single oral doses of the OPs revealed BMD10 values of 1.9 and 0.12 mg/kg (BMDL10 = 1.5 and 0.07 mg/kg) for acephate and chlorpyrifos, respectively. Nevertheless, the dose of chlorpyrifos (3 mg/kg) that induced no clinical signs of overt toxicity significantly reduced both plasma BuChE and motor activities. Therefore, the inhibition of plasma BuChE is not an adverse effect itself but may indicate a potential for the induction of adverse effects by OP toxicity in experimental animal models.

3094 PPARγ Loss Increases Metastasis of HER2+ Breast Tumors

E. D. Lightbody[1], K. M. O'Connell[2], R. E. Rubino[2], A. J. Apostoli[1], M. M. Schneider[1], S. K. SenGupta[1] and C. J. Nicol[1,2]. [1]*Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada and* [2]*Division of Cancer Biology and Genetics, Cancer Research Institute, Kingston, ON, Canada;* [3]*Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada.*

Breast tumours overexpressing epidermal growth factor receptor 2 (HER2+) grow and spread faster than HER2-negative tumours, resulting in poor patient prognosis. We showed peroxisome proliferator-activated receptor (PPAR)γ suppresses environmental carcinogen (DMBA)-mediated breast tumour progression *in vivo*, but the role of PPARγ during HER2+ breast tumourigenesis is unclear. We hypothesized PPARγ loss enhances HER2+ breast tumour progression. Using parental HER2+ (NIC) mice, we generated unique NIC;PPARγKO mice with targeted PPARγ deletion in the same spontaneous HER2+ transformed mammary epithelial cells that drive breast tumourigenesis. Monitored NIC;PPARγKO mice (n=17) have delayed mammary tumour onset but increased HER2+ mammary tumour lung metastasis versus NIC mice. Western blots of NIC;PPARγKO tumours show PPARγ protein decreases as the mammary gland progresses from normal to tumourigenic tissue. Loss of PPARγ protein also inversely correlated with increased HER2 phosphorylation at tyrosine 877 (pY877HER2). HER2 expression was also assessed using immunofluorescent analysis of formalin-fixed, paraffin-embedded breast tumour sections from NIC;PPARγKO and DMBA-induced wildtype (PPARγ-WT) and mammary epithelial HER2Low;PPARγKO mice. HER2 expression was significantly increased in NIC;PPARγKO primary and metastatic tumours, and primary HER2Low;PPARγKO tumours versus PPARγ-WT controls ($p<0.05$). Cell lines established from freshly isolated NIC;PPARγKO lung metastatic tumours, NIC;PPARγKO-lmets, were assessed using scratch wound and boyden chamber assays. Cell migration and invasion were significantly enhanced with EGF treatment, and abrogated with PPARγ activating ligand ($p<0.05$). Together, these data provide the first evidence that PPARγ is a useful prognostic/predictive biomarker for HER2+ breast tumours, and suggest the use of PPARγ ligands may benefit some HER2+ breast cancer patients.

3095 Full Medical Management Supportive Veterinary Care and Survival Following a Single Dose of Irradiation

R. Love[2], K. Thrall[2], R. Manning[2], G. De Los Santos[2], T. Rogers[2], S. Glaza[2], T. Beck[2], H. Tsusaki[2], K. Fukuzaki[2] and R. Nagata[1]. [1]*Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan and* [2]*SNBL USA, Ltd., Everett, WA.*

To create a robust baseline data, define an LD50/60, and to characterize medical measures for animal model development under the FDA Animal Rule (21CR314, subpart 1), well defined veterinary medical treatment regimen and supportive care were provided in animals following irradiation. Forty-eight male rhesus monkeys (4-8 kg) were randomized into six groups of eight per group in a blinded study, and exposed to bilateral 6 MV linear accelerator photon radiation (Varian 600 C/D LINAC) at doses of 6.25 to 8.75 Gy at 0.80 Gy min-1. Animals were evaluated for 60 days for hematology, body weights, body temperature, food consumption, and clinical observations. Full medical management supportive care was provided, with the primary end-point being survival at 60 days post-irradiation. Results: Unsedated daily cageside observations were performed, with periodic sedated detailed clinical observations on Days 1 through 25, and Days 28, 31, 35, 39, 43, 46, 49, 56, and 60, to monitor the clinical condition of each animal. Veterinary care was provided on an individual animal basis. Each animal received full veterinary medical treatment regiment from veterinary staff consisting of analgesics, anti-ulcerative, anti-diarrheals, antibiotics, anti-pyretics, anti-emetics, nutritional and fluid support, and blood transfusion support, as necessary. Hematocrit (HCT), hemoglobin (Hgb), and platelet counts (PLT) were evaluated against a pre-established blood transfusion criterion as follows: HCT≤ 25% over two consecutive hematology time-points, or HCT <20%, or HCT decrease of 10% or move over 2 consecutive hematology time-points, or PLT <2,000/μL, or PLT ≤20,000/μL with Hgb ≤6.7 g/dL. The survival curve demonstrated a dose-dependent effect of irradiation. Twenty-five of 48 animals (52%) succumbed over the dose range from 6.25 to 8.75 Gy by Day 23. The LD30/60 was defined as 6.88 Gy, LD50/60 of 7.43 Gy, and an LD70/60 of 7.98 Gy, and an LD 90/60 was defined as 8.77 Gy with a relatively steep slope of probits per linear dose. No deaths were observed 24 - 60 days post irradiation.

3096 Evaluation of Background Data from CD1 Mice Used in Carcinogenicity Studies

P. Mansell, A. Adamou and G. Hennig. *Charles River, Montreal, QC, Canada.* Sponsor: M. Vezina.

The CD1 mouse has been used in conventional rodent bioassays for the assessment of carcinogenic potential in drug development for several decades. To establish the continuing relevance of the CD1 mouse as an appropriate and stable model for carcinogenicity testing, data collected over a twenty year period from Crl:CD1 mice housed in a conventional non-barrier facility, was evaluated. Primary end points selected included survival, body weight and body weight gain, food consumption and incidence of common neoplastic and non-neoplastic lesions. Dosage routes included oral gavage, dermal, dietary and nose only inhalation. In the majority of studies, mice received a standard rodent diet containing 20% protein fed ad libitum, there was no evidence of any changes in food consumption patterns over the period of investigation when comparing recent studies to studies from the 1990's. Similarly, body weight changes over time were not apparent and this correlated with the previously observed stability in food consumption. There was no evidence of major trends in changes of survival rates over time and introduction of social housing appears to have no influence on survival. Overall survivability at 104 weeks in males was 52% (ranged from 38 to 86%) and 46% (30 to 84%) in females. Neoplastic lesions were commonly observed in several tissue types including adrenal glands, hemolymphoreticular tissue, liver and lung. These tumor types are typical of what has been reported in the literature and indicate a level of uniformity across the strain and there was no evidence of significant increases in any tumor type. The uniformity of the model has been demonstrated over a prolonged time course for carcinogenicity testing demonstrating that it remains a stable model in support for successful drug registration.

3097 Comparison of Historical Control Parameters Derived from Carcinogenicity Studies Conducted in CD®IGS Rats and CD-1®IGS Mice Fed 5002 or 5CR4 Certified Diets

M. A. Morse, B. Jacob and A. M. Sargeant. *Charles River Laboratories, Spencerville, OH.*

LabDiet certified rodent 5002 and 5CR4 diets are two diets used in GLP carcinogenicity studies. One of the major differences between the two diets is the protein content (5002: 20% protein; 5CR4: 14% protein). To assess possible differences in the effects of these diets on carcinogenicity study endpoints, historical control parameters from carcinogenicity studies conducted within the last 5 years using 5CR4 diet and within the last 10 years using 5002 diets were compared in male and female CD®IGS rats (Crl: CD(SD)) and CD-1®IGS (Crl: CD1(ICR)) mice. Among the parameters evaluated were survival, tumor incidence, and the incidence of chronic progressive nephropathy in rats and mice. Survival to scheduled euthanasia was comparable in female CD®IGS rats or CD-1®IGS mice of either sex fed the 5CR4 or 5002 diets, whereas male CD®IGS rat survival was significantly greater ($p < 0.01$) in rats fed the 5CR4 diet (44%) versus the 5002 diet (33%). The tumor profiles for each diet were generally comparable in both CD®IGS rats and CD-1®IGS mice. The most notable difference between the two diets was in the incidence of chronic progressive nephropathy. The incidence of nephropathy was significantly lower with the 5CR4 diet than with the 5002 diet in rats (males: 66% vs. 83%; females: 20% vs. 44%) and mice (males: 43% vs. 67%; females: 38% vs. 56%). In conclusion, although tumor profiles were generally comparable between animals fed 5CR4 or 5002 diets, the 5CR4 diet was associated with greater survival in male rats and with a lower incidence of chronic progressive nephropathy in male and female rats and mice compared to the 5002 diet. Further investigation is needed to determine the influence of the two diets on the severity of nephropathy and the role of nephropathy in survival.

3098 Comparison of Routine Clinical Pathology Parameters of Cynomolgus Monkeys Maintained in Different Housing Conditions

L. Guo, S. McPherson, C. Zheng, S. Chou and M. Chen. *Toxicology, Wuxi AppTec, Suzhou, China.*

Social housing of nonhuman primates used in biomedical research is being implemented around the world as one of the approaches to improve their psychological wellbeing. Given the constrains of available caging, housing space, and available candidates for social housing, many research institutions use pair- or trio-housing arrangements to fulfill regulatory and programmatic requirements of social housing. Beneficial effects can include ability to demonstrate species-typical behaviors, better able to cope with common laboratory stressors which result in fewer maladaptive behaviors, and presence of a balanced temperament in compatible groups. On the other hand, as with other forms of enrichment, the concern over social housing as a potential research variable should be assessed. A comparison of common hematology and clinical pathology data between single-housed and pair- or trio-housed cynomolgus macaques is made. The results from the two data sets were analyzed using the socially housed Cynomolgus Monkeys as reference control point. Analysis of the hematology data showed slightly higher white blood cell count (WBC), including absolute count of neutrophils (NEUT), monocytes (MONO), and eosinophils (EOS, for males only) and slightly lower reticulocyte count (RET) in both sexes of singly housed monkeys when compared to the socially housed monkeys. Differences in clinical chemistry were observed for singly housed male monkeys included a reduced alanine aminotransferase (ALT), triglycerides (TG), potassium (K), and globulin (GLB) when compared to the socially housed monkeys. Slight increases in total bilirubin (TBIL) and decreased total cholesterol (TCHO) were noted for the singly housed female monkeys relative to the socially housed monkeys. Whilst the data showed no major differences in parameters we would strongly recommend that separate data sets are maintained for the interpretation of clinical pathology data. In addition, this data supports the practice of pair- and tri-housing as an acceptable method to maximize psychological wellbeing of cynomolgus macaques without introducing confounding variables in drug safety testing studies.

3099 Repeated Intracerebroventricular Administration via Implanted Cannula with Continuous CSF Collection via Implanted Intrathecal Access Port in Cynomolgus Monkeys

S. Watanabe[2], B. Megrath[2], R. Love[2], P. Franklin[2], G. De Los Santos[2], T. Rodgers[2], S. Glaza[2], T. Beck[2], K. Fukuzaki[2] and R. Nagata[1]. [1]*Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan and* [2]*Scientific Services, SNBL USA Ltd., Everett, WA.*

Intracerebroventricular (ICV) drug administration is a method that bypasses the blood-brain barrier and other mechanisms that limit drug distribution into the brain and intrathecal (IT) area. The combination of an implanted ICV cannula and an implanted IT catheter with an access port provides the means for repeated ICV dose administration followed by continuous monitoring of drug concentration or target protein concentration in cerebrospinal fluid (CSF) without the need for chemical sedation. Ten cynomolgus monkeys, previously implanted with IT catheters, were surgically implanted with ICV cannula stereotaxically. The initial target of the cannula placement was +16.4mm anterior to the interaural line, +/- 2.0 mm lateral of the midline, and 15.8 mm beneath the level of the dura mater. Placement of the cannula into the ventricular space was confirmed with artificial CSF (aCSF) and verified radio-graphically using a fluoroscope with contrast media (Omnipaque). While keeping the cannula in position, anchoring screws and cement were applied. Post-surgery ICV cannula and IT catheter maintenance was performed once weekly to verify patency and assess the overall condition of the animals prior to dose administration. Eight animals with patent catheters were dosed with aCSF into the ventricular system via infusion through the implanted cannula for multiple days and CSF samples were collected via the IT access port at various time points. Multiple ICV dose administrations were well tolerated by all animals and IT access ports in 7 of 8 animals remained patent during the study. In conclusion, this ICV cannula and IT access port technique has proven useful in repeated ICV dose administration and continuous CSF sampling and could be considered as Central Nervous System dose and monitoring model in cynomolgus monkeys.

3100 Development and Validation of a Bleeding Time Assessment Model in the Non-Human Primate

J. Sheehan. *Surgical Services, Envigo, East Millstone, NJ.* Sponsor: T. Ramani.

Bleeding time assessments are often used in drug development to determine the efficacy, safety and dose response of anantcoagulant. Whereas many animal models have been described in literature, there are surprisingly few studies on suitable methodologies in non-human primates. The method chosen must be appropriate for the species as well as the mode of action of the experimental anticoagulant and control substances. This presentation will describe the technical aspects and challenges involved in validation of a bleeding time assessment model in non-human primates using both a skin template and internal (organ) bleeding techniques. Consistent skin bleeding could be obtained using a modified, yet simple, template method. Anticoagulants that don't affect platelet function (e.g. heparin) did not markedly affect skin bleeding time although post-recovery effects (hematomas and re-bleeds) were

evident at high doses. Internal bleeding was most successfully reproduced using standardized liver cuts but was less successful in the kidney and mesenteric artery. The liver bleeding model was fully validated for use as a bleeding time assessment model in the non-human primate to evaluate anticoagulants.

3101 Addition of Left Ventricular Pressure Measurement to a Cardio-Respiratory Model, Using Telemetry and Plethysmography in the Conscious Rat

S. Beaudoin, C. Williams, V. Milner, K. Melliti and K. Meecham. *Pharmacology, Envigo, Huntingdon, United Kingdom.* Sponsor: D. Mitchell.

Models allowing the combined assessment of cardiovascular and respiratory effects in a single animal are considered optimal with respect to reducing both animal use and compound requirements. The aim of this project was to examine the feasibility of also adding left ventricular pressure (LVP) measurements to a combined cardiovascular and respiratory model using telemetry and plethysmography in conscious Han Wistar (Crl:WI(Han)) rats. Effects on respiratory rate (RR), tidal volume (TV), minute volume (MV), arterial blood pressure (systolic, diastolic and mean (MAP)), heart rate, left ventricular pressure parameters (LVSP, LVEDP, dP/dtmax and dP/dtmin) and body temperature were assessed in this project (effects of selected reference compounds compared with vehicle control), along with monitoring of the long-term stability of the LVP signals. Theophylline (100 mg/kg, p.o.) was selected to assess respiratory effects and exhibited the expected respiratory stimulant effect (MV, +300%, RR, +282% and TV, -30%; all $p<0.001$) with concurrent tachycardia (HR, +65%, $p<0.001$) and increases in dP/dtmax (+94%, $p<0.001$). Increases in body temperature were also observed (+1.2°C, $p<0.001$). Verapamil (30 mg/kg p.o.) was selected to assess cardiovascular effects and induced the expected reduction of MAP (-21%, $P<0.001$), with resultant tachycardia (HR, +40%, $p<0.001$) and also induced a long-lasting reduction of dP/dtmax (-43%, $p<0.001$). LVP signals remained stable for at least 50 weeks post-surgery. This project demonstrates that addition of LVP to a combined cardio-respiratory model can provide further detailed information on cardiovascular function and has the potential to allow screening for effects on cardiac contractility over long periods.

3102 Short-term Carcinogenicity Study on C57BL/6J-Tyr[X]-rasH2 (Albino-rasH2) Mice, a Novel Strain for *In Vivo* Tumor Imaging

T. Mizushima[1], K. Urano[1], R. Inoue[1], M. Yasuda[1], M. Goto[1], T. Kagawa[1], T. Kadonosono[2], T. Kuchimaru[2] and S. Kizaka-Kondoh[2]. [1]*Central Institute for Experimental Animals (CIEA), Kawasaki, Kanagawa, Japan and* [2]*Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Kanagawa, Japan.* Sponsor: K. Urano.

CBYB6F1-Tg(HRAS)2Jic (rasH2) mouse, which was introduced HRAS(c-Ha-ras) gene, has high susceptibility to human carcinogens and has been used in short-term carcinogenicity studies for new drugs. In this study, we investigated the carcinogenic susceptibility of further genetically modified rasH2 mice for a novel carcinogenicity evaluation system using *in vivo* optical imaging technique. Since the color of hair coat largely affects the sensitivity on *in vivo* whole-body imaging, we introduced a tyrosinase mutant gene (Tyr[X]), which caused albinism, into rasH2 mouse to establish C57BL/6J-Tyr[X]-rasH2 (albino-rasH2) mouse. To evaluate the carcinogenic susceptibility of albino-rasH2 mouse, a 26-week short-term carcinogenicity study was performed using a standard positive control agent, N-methyl-N-nitrosourea (MNU). Thirty mice of each sex, were divided into a vehicle group (Citrate buffer, pH 4.5, 10 ml/kg) and a MNU (75 mg/kg) group (15 mice/ sex / group). Vehicle or MNU was once injected intraperitoneally at 7 weeks of age. During 26-week observation, MNU-induced tumor death was observed at 10 weeks after administration and thereafter in each sex. The final survival rate in male and female was 13.3% and 20.0% with MNU group, whereas 93.3% of mice had survived in both sexes in vehicle group. MNU-induced tumors in albino-rasH2 mice were found in forestomach, skin, lung, thymus and spleen, which was similar to that of the original rasH2 mice. On the other hand, a few mice in vehicle group died caused by lymphoma, which was rare in the original strain. Although further data analysis and more investigation using other carcinogenic agents should be required, these results suggest that the albino-rasH2 mouse has high potency in the carcinogenetic susceptibility comparable to that of the original rasH2 mouse.

3103 Comprehensive Vital Systems Monitoring in the Rabbit

M. Horsmon[2], N. Vincelli[1] and R. Kristovich[2]. [1]*EXCET, Gunpowder, MD and* [2]*US Army ECBC, Gunpowder, MD.* Sponsor: H. Salem.

The present study investigates the feasibility of applying two implantable telemetry devices capable of providing data on animal activity, core body temperature, blood pressure (BP), electrocardiogram (ECG), electroencephalogram (EEG), and impedance-based respiratory parameters in the rabbit. Six New Zealand white rabbits were implanted with two telemetric devices one device is placed in the abdominal cavity while the other is placed subcutaneously between the scapulae. The first task was to develop an optimal implantation technique that yields calibrated tidal volume (Vt) measurements that are within 10% of those obtained simultaneously from a pnemotachograph (PNT), low noise ECG, stable BP, and regular EEG. The second task was to challenge with a known respiratory stimulant and a seizure inducer (doxapram HCl, 5.0mg/kg I.V. and bicuculline HCl (0.2mg/kg I.V. respectively) to assess linearity of the calibration across a range of Vt and confirm EEG electrode placement captures generalized seizure activity. Of the three impedance electrode placements attempted, only one resulted in calibrations consistently under 10% error. Optimal impedance electrode placement results in calibrated Vt measurements within 1.7% (±1.6%) of those obtained from a PNT during normal tidal breathing, 6.0% (±3.6%) following doxapram HCl injection and 7.3% (±4.4%) following saline injection. Vt range for normal tidal breathing and saline injection was 9-15mL, and following doxapram injection was 25-30mL. Similar error ranges were associated with derived flow parameters. Increases in mean BP of 25.0mmHg (±6.82mmHg) and decreases in heart rate of 56.3bpm (±6.82bpm) were associated with doxapram injection only. In all cases, the two electrode EEG placement was able to detect generalized seizure activity induced by bicuculline administration. No departure from normal body temperature was observed in any group. The development of this model offers a solution to monitoring vital organ system function the conscious rabbit.

3104 Nude Female Mice Catheterised with the Pinport-in-Tail Cuff System, Allowing Disconnection of Tether (Potential Group Housing) in Intermittent Infusion Studies for up to 108 Days

H. van Wijk[2], U. Wirnitzer[1] and E. Hartmann[1]. [1]*Bayer Pharma, Wupperthal, Germany and* [2]*Covance Laboratories, Harrogate, United Kingdom.* Sponsor: A. Jackson.

The nude mouse has been used to generate human tumour xenografts, for testing anti-tumour efficacy of new anti-cancer agents. Intravenous administration of compounds can be given as a slow bolus injection (tail vein) or by an implanted femoral catheter with tail cuff exteriorisation. Infusion in permanently tethered mice should be selected for studies where the compound will be administered either continuously or for repeated intermittent doses for between 7 and 14 days where the duration of infusion is greater than 5 minutes. When continuously tethered, the physical effect of the tail cuff fixation limits studies to 28 days due to wire passed through the tail to secure the cuff, resulting in tissue damage. This study, performed in Bayer, compares histopathology findings of 17 tail cuffed mice, continuously tethered for 11-51 days (Gp A) with 12 mice tethered only transiently via an in-line pinport-in-tail cuff system for 21-108 days, for administration/flushing (GpB). During non-administration periods mice were disconnected. Microscopic evaluation was carried out at set points of the catheter course (eg. vena cava, femoral vein, tail before tail cuff, tail area covered by tail cuff, tail after tail cuff).The microscopic findings in and around the femoral vein in response to the presence of catheter indicated a slight improvement in Gp B of the mean severity score: phlebitis/periphlebitis (88% Gp A, 58% Gp B); inflammation (100% - Gp A, 75% Gp B) muscle fibre degeneration (29% Gp A, 0% Gp B). No difference was observed in thrombus in the vessel lumen of the vena cava, (47% Gp A, 50% Gp B). In conclusion, many microscopic findings at the tail in Gp B were decreased in incidence and severity grading. This indicates that intermittent infusion can be extended when using the pinport-in-tail cuff system which allows for disconnection of the mice during periods of no administration.

3105 Gastroinstestinal Motility: Motility and Motor Migrating Complex (MMC) Evaluations in Rats, Dogs and Non-Human Primates

R. Kubaszky[1], S. Authier[1], H.-M. Tang[2], A. Ascah[1], M. Pouliot[1], S. ABTOUT[1], K. Bujold[1], R. Forster[1], E. Troncy[1] and M. Pugsley[1]. *[1]CiToxLAB North America, Laval, QC, Canada and [2]Novartis Pharmaceuticals Corporation, One Health Plaza, East Hanover, NJ.*

Drug-induced effects on gastrointestinal motility are observed with a number of approved drugs but a relatively narrow range of non-clinical assays is available in drug development. Fluoroscopic video imaging was used to assess oesophageal and gastric motility in rats using a buccal iodixanol radio-opaque meal. In Beagle dogs and cynomolgus monkeys, this methodology was used to monitor gastric and intestinal motor migrating complexes and gastric emptying times using 10x2 mm radio-opaque beads and/or barium solution. In dogs, gastric pH was sequentially measured from fasted animals or after a wet food meal. In rats, baseline oesophageal transit time was 2.23 ± 0.54 sec and a significant increase was induced by morphine (20 mg/kg). Baseline gastric motor migrating complexes (MMC) in non-human primates during the day was 2.82 ± 0.50 contractions per minute with an average gastric emptying time of a semi-solid meal at 206.3 ± 63.9 min. Mosapride (1 mg/kg) decreased gastric and intestinal emptying time. Morphine (2 mg/kg and 10 mg/kg) decreased the number of gastric contraction per minutes in cynomolgus monkeys. In dogs, feeding was shown to significantly increase gastric emptying time compared to the fasted state but also significantly decreased gastric pH for a period of 5 hours. MMC present circadian cycle patterns and controlling for day time is an important factor in functional evaluations. Large animals presented individual MMC patterns and comparison of drug-treated sessions with a time-matched control period from the same animal yielded optimal sensitivity. In large animals, a cross-over study design is preferable whenever possible.

3106 An Inhalational Model for the Toxic Industrial Chemical Cyanide in Awake Swine

M. Perry, J. Plahovinsak and M. Brittain. *Battelle, Columbus, OH.*

There is recent concern over the terrorist use of toxic industrial chemicals (TICs) because of the large quantities available as well as their highly toxic nature. The most probable route of exposure to many TICs, including cyanide, is via inhalation. Effective testing of medical countermeasures is best performed using a relevant animal model via relevant route of exposure. The objective of this study was to develop an inhalation exposure system for evaluating hydrogen cyanide (HCN) lethality in domestic swine that could be easily transitioned to other TICs of interest. A custom fabricated exposure system was used to deliver controlled HCN vapor challenges generated by mixing HCN gas with ambient air. Animal breathing parameters were monitored real-time via an in-line pneumotach, pressure transducer, signal amplifier, and pulmonary analysis software. Unanesthetized female Yorkshire swine, Sus scrofa domesticus, weighing 20 ± 5 kg were quarantined and then acclimated to a restraint device, exposure cone, and head harness over a 3-week period. Five swine were exposed to HCN concentrations ranging from 260 to 986 ppm. Pulmonary function (respiratory rate and tidal volume) and clinical observations were continuously monitored during the exposure. Following challenge, clinical and behavioral monitoring was performed at 5, 10, 15, 30, 60 min, and 24 hrs post-exposure for survivors. During exposure to varying levels of cyanide, respiration rates and tidal volumes increased, while animals were observed to vocalize, become agitated, salivate, exhibit respiratory distress, and apnea. Two of the five animals survived to 24 hrs and were observed as normal throughout. The remaining three animals succumbed to cyanide intoxication with death occurring within 10 min post-challenge. Overall, a dose-dependent response was observed with the LD50 for inhaled swine estimated to be between 2.45 and 2.57 mg/kg. Additional testing would be required to accurately determine the LD50 and confidence intervals. Historically, inhalation exposure to an unanesthetized large animal has proven to be difficult. The development of this inhalational awake swine model for cyanide exposure has the ability to be easily transitioned to numerous other TICs of concern (e.g. aniline, chlorine, phosgene), providing a relevant large animal exposure model for therapeutic testing against various TICs.

3107 Serum Chemistry, Hematology, Urinalysis, and Flow Cytometry Evaluation and Characterization in Juvenile and Adult Beagle Dogs

J. Toot[3], R. Tadagavadi[3], C. Synan[2], P. Tarantino[1], S. Davis[3], J. England[3] and J. Figueiredo[3]. *[1]Idera Pharmaceuticals, Inc., Cambridge, MA; [2]Sunovion Pharmaceuticals, Inc., Marlborough, MA and [3]WIL Research, Ashland, OH.*

Although clinical pathology and flow cytometry evaluations are being conducted on a relatively routine basis in adult dogs as part of general toxicology study packages, their utility as endpoints in juvenile toxicology studies has been limited due to the availability of historical control data. Therefore, the purpose of this study was, in part, to evaluate and characterize standard clinical pathology and flow cytometry endpoints in beagle dogs from juvenile through adulthood. Selected endpoints were evaluated from approximately 8 animals/sex beginning on postnatal (PND) 32 for clinical pathology and PND 49 for flow cytometry evaluations up to PND 238 covering 8 or 7 evaluation periods, respectively. Study animals were assigned to clinical pathology and flow cytometry endpoints across litters, in order to avoid litter bias. Clinical pathology evaluations consisted of serum chemistry (e.g., albumin, total protein, albumin/globulin ratio, total bilirubin, creatinine, ALP, ALT, AST, GGT, glucose, cholesterol, electrolytes, CK, LDH, etc.), hematology (e.g., RBC, HGB, MCT, MCV, MCH, MCHC, platelet, leukocyte count, RDW, HDW, etc.), coagulation parameters (PT, APTT, and fibrinogen) and urinalysis (e.g., color, clarity, SG, pH, glucose, ketones, WBC, RBC, electrolytes, creatinine, etc.). Flow cytometry evaluations consisted of CD3+ T cell, CD3+CD4+ T helper cell, CD3+CD8+ T cytotoxic cell, CD3-CD21+ B cell, and CD3-CD14+ monocyte assessments. Over the age range evaluated, there was an age-related decrease of reticulocyte counts, HDW, ALP activity and phosphorus concentration along with an age-related increase in RBC counts, MCHC, percent hematocrit and hemoglobin, creatinine, total protein, albumin, and globulin concentrations. The remaining serum chemistry, hematology, coagulation, urinalysis, and flow cytometry parameters measured in male and female beagles were generally consistent and did not show remarkable changes with age. The results of this study provide historical control data to be used in the interpretation of clinical pathology and flow cytometry related data for a range of developmental periods in the beagle dog.

3108 Changes in Blood Cholinesterases and Heart Rate Recovery Following Subchronic Pyridostigmine Exposure in Rats

M. Bharadwaj, L. K. Maxwell, M. Davis and C. N. Pope. *Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK.*

Sympathetic overactivity and parasympathetic withdrawal indicate profound dysregulation of autonomic control in congestive heart failure (CHF). Rapid heart rate deceleration after exercise, or heart rate recovery (HRR), is a simple method to assess these changes in cardiac sympathovagal function. HRR is a measure of reactivation of the parasympathetic system. Acetylcholinesterase (AChE) inhibitors such as pyridostigmine bromide (PYR), increases the parasympathetic tone in the heart and may enhance HRR in CHF patients. We developed a rat exercise model of HRR to test our hypothesis that subchronic administration of PYR enhances HRR after moderate exercise by increasing the parasympathetic tone. Sixteen male, Sprague Dawley rats underwent surgery for implantation of radiotelemetry transmitter devices for measurement of heart rate (HR). After recovery, the rats were randomly allocated into either a PYR or a control (CTL) group (n=8). PYR rats received 0.14 mg/mL of PYR dissolved in drinking water for 29 days, whereas the remaining rats received only water. All rats were moderately exercised on days 0, 7, 14 and 28. HR was recorded during and after exercise. Intraperitoneal injections of atropine sulfate (2 mg/kg) and propranolol (4 mg/kg) were used to evaluate the long term effects of PYR on cardiac vagal and sympathetic tones on days 27 and 29. Both red blood cell and plasma AChE activity was inhibited by >60% compared to the baseline values ($p<0.001$). HRR was significantly higher in the PYR rats on days 7, 14 and 28 ($p=0.003$, <0.001, 0.03). Vagal tone (measured as HR after atropine treatment) was increased in PYR rats which was shown by a 17% decrease in HR compared to only 3% in CTL rats ($p<0.001$). In contrast, sympathetic tone (measured as HR after atropine treatment) and basal HR were unaffected by PYR. Intrinsic HR (measured after combined atropine-propranolol treatment) was similar on day 27 but was higher (338±6 vs. 376±8 bpm) in the PYR rats on day 29 ($p=0.002$). Together, these data support our hypothesis that PYR enhances HRR by increasing cardiac vagal tone. Post-exercise HRR can be used in toxicology or pharmacology studies to assess parasympathetic tone in rats.

3109 *In Vitro* Renal Cytotoxicity Induced by Putative Metabolites of 3,5-Dichloroaniline

C. R. Racine, D. Ward, C. Tyree, D. Pope, J. Sharp, D. Anestis and G. O. Rankin. *Department of Pharmacology, Physiology, and Toxicology, Marshall University, Joan C. Edwards School of Medicine, Huntington, WV.*

The nephrotoxic potential of 3,5-dichloroaniline (3,5-DCA) has been well established in rats both *in vivo* and *in vitro*. Previous studies have explored the role of renal biotransformation in 3,5-DCA-induced nephrotoxicity *in vitro*. When pretreated with various biotransformation enzyme inhibitors, 3,5-DCA -induced cytotoxicity was significantly attenuated. These studies suggested that 3,5-DCA is biotransformed via multiple pathways to toxic metabolite(s) in rat isolated renal cortical cells (IRCC). The current study was designed to explore the nephrotoxic potential of three putative metabolites [2-amino-4,6-dichlorophenol (2A4,6DCP), 3,5-dichlorophenylhydroxylamine (3,5-DCPHA), and 3,5-dichloroacetanilide (3,5-DCAA)] of 3,5-DCA in IRCC. Briefly, IRCC (~4.1 million cells/ml; 3 ml) from male Fischer 344 rats were exposed to various concentrations [2A4,6DCP, 3,5-DCAA (0.5-1.5 mM); 3,5-DCPHA (0.25-1.0 mM)] of each putative metabolite or DMSO for 60 or 90 min. Cytotoxicity was determined by lactate dehydrogenase (LDH) release assays. Results showed that there were no significant changes in LDH release following exposure to 3,5-DCAA at any time point or concentration tested. On the other hand, 2A4,6DCP was a nephrotoxicant at all concentrations, but only after 90 min exposure. In contrast, 3,5-DCPHA significantly increased LDH release at concentrations of 0.5 mM or greater after 60 min and at all concentrations tested after exposure for 90 min. These results, along with previous studies, suggest that the putative metabolites 3,5-DCPHA and/or 2A4,6DCP, but not 3,5-DCAA, may contribute to 3,5-DCA induced nephrotoxicity. (Supported in part by NIH grant P20GM103434).

3110 Nephrotoxicity of Engineered-nanoparticles: Exposure Effects of Silver Nanoparticles in a Kidney Microphysiological System

K. P. Van Ness[2], E. J. Weber[2], T. Neumann[1] and E. J. Kelly[2]. [1]*Nortis, Inc, Seattle, WA and* [2]*Pharmaceutics, University of Washington, Seattle, WA.*

The number and diversity of metal-based engineered nanomaterial (ENM) forms has increased dramatically while toxicology testing of many of these nanomaterials has not been rigorously evaluated. *In vivo* toxicology testing using traditional laboratory test species for the thousands of ENM forms is not feasible thus requiring the development of *in vitro* systems to rapidly screen toxicity. We have developed an organotypic microfluidic model that utilizes the Nortis microphysiological (MPS) system that accurately reflects human renal physiology. We conducted a renal ENM exposure scenario by perfusing 20 nm citrate silver nanoparticles through a tubule of polarized primary human proximal tubule epithelial cells (PTEC) using a physiologically-relevant 3-D configuration and flow rate. We exposed human PTECs cultured in MPS devices to 0, 5 and 25 ug/mL to silver nanoparticles for 48 hours and evaluated device effluents for the cell-based marker of cellular injury kidney injury marker (KIM-1). Ongoing analyses includes the assessment of cellular ultrastructures by electron microsopy, Adverse Outcome Pathway (AOP) determinations using RNA-seq transcriptomic analysis and immunocytochemistry (ICC) fluorescent imaging for KIM-1 and heme-oxygenase (HO-1), an oxidative stress marker. After 48 hours of silver nanoparticles exposure, minimal PTEC toxicity was observed with no gross changes in cell morphology and no evidence of KIM-1 induction by ICC. However, a modest dose-dependent increase in HO-1 cell associated fluorescent signal was observed suggesting that silver nanoparticle exposure to PTEC can cause oxidative stress.

3111 A Novel Microfluidic Device to Model Human Kidney Disease and Toxicity

C. Sakolish, M. Chambers and G. Mahler. *Biomedical Engineering, SUNY Binghamton, Binghamton, NY.*

Physiologically-based cell models could potentially be utilized to accurately model human systems and allow for more directed pharmaceutical testing and toxicology studies than what is being achieved with cell cultures or non-human mammalian models. The proximal tubule region of the kidney is responsible for up to 90% of renal toxicity cases, making it an attractive target. The goal of this project to develop a multichannel microfluidic device with incorporated shear stress and glomeruluar filtration that models the human proximal tubule in both healthy and diseased states. Multi-channel microfluidic devices were designed with an 8nm glomerular filter, followed by 2 channels separated by a fibronectin-treated cell support (0.4µm pore polycarbonate). HK-2 cells (human proximal tubule) were cultured in DMEM/F-12 supplemented with 10% FBS at 37°C. Prior to experimentation, cells were seeded onto the device support (fluidic), or onto Transwells® (static) at 100,000 cells/cm2 and allowed to grow for a period of 24 hours before the introduction of shear stress (0.8 dyne/cm2). Cells were monitored under normal conditions as well as disease states such as low/no flow, high-glucose induced fibrosis, bacterial infection (E-coli) and renal calculi (1.46mg/mL calcium oxalate in media). Protein transport was observed through the addition of FITC-BSA (0.4mg/mL) to media while culturing under these conditions. Cell function was assessed through the use of immunohistochemistry staining (clathrin, β-catenin), monitoring of LDH release and IL-7 and IL-8 ELISA. Fluidic cultures displayed a 13-fold increase in the expression of active transporter protein, clathrin (3.16 ± 0.69% positive staining compared to 40.69 ± 8.38%), and this transport was also confirmed through a significant increase in transport of fluorescently labelled BSA in fluidic conditions against static controls. This low degree of transport in static cultures may explain why traditional culturing methods fail to detect toxicity in pre-clinical trials. Additionally, changes in cell morphology were observed in the microfluidic device compared to static controls. Cells elongated in the direction of shear stress as would be observed *in vivo*, but in static cultures, grew together chaotically. Finally, results of disease modeling indicated a potential protective effect of fluid shear stress on the cells compared to static controls. This device also has applications in toxicity studies, which will be further investigated.

3112 Investigating the Solute-dependent Transport of Oxalate Ion and the Kinetics of Calcium Oxalate Monohydrate Crystal Internalization

E. Luttrell-Williams and K. McMartin. *Pharmacology, Toxicology & Neuroscience, LSU Health Sciences Center - Shreveport, Shreveport, LA.*

Thousands of ethylene glycol ingestions are reported in the US each year, with poisonings producing metabolic acidosis and acute kidney injury. The renal damage produced by ethylene glycol poisoning has been associated with the accumulation of calcium oxalate monohydrate (COM) crystals in the proximal tubule. The kinetics of COM uptake and of oxalate ion transport have not been fully characterized in human proximal tubule (HPT) cells, and could have major implications for the observed COM retention. Additionally, aluminum citrate offers protection against ethylene glycol exposure in rats through an unknown mechanism. For these studies, the kinetics of COM internalization and solute-dependent oxalate ion transport in primary HPT cells were characterized. Electron microscopy was utilized to assess COM adhesion and uptake for up to 4 h, with and without aluminum citrate, and at 1 (nontoxic) and 5 (toxic) mmol/L concentrations of COM. Cells grown on semi-permeable inserts were used to examine radiolabeled oxalate ion uptake from both the apical and basolateral directions over the course of 4 h at sub-toxic concentrations, in the presence or absence of sulfate. The findings suggest that, while COM adhesion is rapid, internalization is only initiated after several hours, with only early endosomes visible for internalized crystals by 4 h. Internalization was reduced by aluminum citrate. Ion uptake results suggest that the apical oxalate uptake is significantly increased by sulfate availability, with minimal change in basolateral uptake. These results indicate that proximal tubule cells can reabsorb luminal oxalate crystals as well as the oxalate ion.

3113 Drug-Induced Kidney Injury in a High-Throughput Microfluidic Culture of Human Renal Cell Line CIPTEC

M. J. Wilmer[4], T. Nieskens[4], J. Vriend[4], C. M. Schophuizen[4], P. Vulto[1], H. Lanz[1], L. Suter-Dick[2] and R. Masereeuw[5]. [1]*Mimetas B.V., Leiden, Netherlands;* [2]*University of Applied Sciences Northwestern Switzerland, Muttenz, Switzerland;* [3]*Department of Pediatric Nephrology, Radboudumc, Nijmegen, Netherlands;* [4]*Department Pharmacology and Toxicology, Radboudumc, Nijmegen, Netherlands and* [5]*Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands.* Sponsor: F. Russel.

Background: Reliable prediction of drug-induced kidney injury (DIKI) is a major issue during drug development. The human conditionally immortalized proximal epithelial cell (ciPTEC) cultured in a physiological relevant environment in a multi-compartmental microfluidic titerplate (OrganoPlateTM) can improve DIKI-prediction in early toxicity screening. Method: Gene expression of anionic and cationic drug transporters in ciPTEC was analyzed in static conditions and under fluidic shear stress (FSS). Upon recombinant expression of organic anion 1 and 3 (OAT1/3), ciPTEC were exposed (24hr) to nephrotoxic antivirals adefovir, cidofovir and tenofovir and anti-neoplastic cisplatin. Cell viabil-

ity was assessed by NucBlue Live and Propidium Iodide (PI), or via an MTT assay. Results: Endogenous expression of drug transporters OCT2, Pgp, BCRP and MATE-2k were increased when cultured under FSS (≥ 2-fold). Functionality of transfected OAT1 and OAT3 was confirmed by fluorescein uptake (Km: 0.8±0.1 and 3.7±0.5 μM, respectively), which was inhibited by probenecid (IC50: 12.7±0.5 and 1.9±0.6 μM, respectively). Antiviral induced toxicity was only observed in ciPTEC-OAT1 (IC50 determined by MTT: adefovir 23±4; cidofovir 71±34 and tenofovir 42±8 μM), while all ciPTEC lines were sensitive to cisplatin (IC50 ciPTEC: 80±33; ciPTEC-OAT1: 70±7 and ciPTEC-OAT3: 121±9 μM). Image analysis of ciPTEC-OAT1 cultured in the OrganoPlate™ and exposed to cisplatin resulted in an IC50 of 25 μM. Conclusion: Culturing ciPTEC in the OrganoPlate™ under FSS stimulates expression of drug transporters leading to a physiologically-relevant system suitable for *in vitro* prediction of DIKI. Expression of OAT1/3 in ciPTEC also enables the screening of anionic drugs.

3114 Kidney Proximal Tubule Cells and Cardiomyocytes as Human Cellular Models to Investigate Statin Exposures for Subsequent Assessments of Nephro- and Cardio-Protection

A. Atilano-Roque and M. S. Joy. *University of Colorado, Skaggs School of Pharmacy & Pharmaceutical Sciences, Aurora, CO.*

Statins are prescribed for their renal- and cardio- protective roles in chronic kidney diseases (CKD). However, there is limited published information describing mechanisms for how statins are transported in kidney and heart tissues. This is particularly relevant in CKD since there can be modulations in filtration, secretion, and reabsorption pathways in kidney as well as competition for transport secondary to uremic toxins. Optimized human cellular models to assess statin cellular exposures and outcomes in kidney and heart would have value toward predictions of tissue exposures and outcomes in humans with CKD. Primary human proximal tubule cells (hPTC) and primary human cardiomyocytes (hCM) were obtained commercially and seeded onto 6-well transwell plates at a pre-determined density. The cells were grown for 7-10 days and transepithelial electrical resistance (TEER) measured. Cells were exposed to simvastatin (0-20 μM) for 24 h and TEER, transport (transcellular vs. paracellular), and gene expression was assessed. hCMs failed to establish tight junctions and polarity as measured by TEER and ZO-1 immunofluorescence staining. hPTC polarity was confirmed by TEER (140-148 Ω*cm2) and formation of tight junctions by ZO-1 immunofluorescence. Paracellular transport of Lucifer Yellow increased with doses of simvastatin in hPTCs. The presence of a targeted kidney uptake (OATP4C1) and efflux (P-gp) transporter and a heart transporter (OATP3A1) was confirmed. To characterize net transporter function, [3H]Simvastatin was added in separate experiments to the basolateral (B) or apical (A) side of hPTCs and hCMs and transport detected on the opposite side. Transport of [3H]Simvastatin from B→A and from A→B directions suggest functional OATP4C1 and P-gp activity. Additionally, transporter activity assessments with digoxin, estrone-3-sulfate, and benzylpenicillin will be studied with inhibitors. In conclusion, hPTCs and hCMs are *in vitro* models for investigating human kidney and heart exposures to statins.

3115 Developing a More Clinically Relevant Mouse Model of Nephrotoxicity

C. Sharp[3], M. Doll[3], T. Dupre[3], P. Shah[3], S. Marimuthu[3], D. Siow[3], J. Megyesi[1], G. Arteel[3], L. Beverly[3] and L. Siskind[3]. [1]*Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR;* [2]*Medicine, University of Louisville, Louisville, KY and* [3]*Pharm/Tox, University of Louisville, Louisville, KY.*

Kidney injury is the most critical dose-limiting toxicity in cancer patients treated with a variety of chemotherapeutics, including cisplatin. In fact, 30% of patients administered cisplatin develop acute kidney injury (AKI), requiring the next dose to be withheld or reduced, leading to a less effective treatment. Patients who suffer from AKI have significantly higher rates of chronic kidney disease (CKD) as compared to the general population. The nephrotoxicity of cisplatin has been studied extensively with rodent models that utilize a single, high-dose of cisplatin that results in death of the animal after a few days. Thus, long-term studies of kidney repair, recovery, and function have not been possible. In the clinic, cisplatin is administered to patients as several lower doses spaced a few weeks apart to allow the kidney time to recover. We have developed a new model of cisplatin-induced kidney injury that more accurately reflects the clinical dosing and timing of treatments, and allows for the analysis of cisplatin injury as well as the long-term effects on renal function. This new model of cisplatin caused inflammation, interstitial fibrosis, and accelerated aging in the kidney as a result of the repeated injury from the multiple, low doses of cisplatin. Data suggests that inflammation and fibrosis, as opposed to apoptosis and necrosis, are perhaps more physiologically relevant cellular process responsible for not only the initial cisplatin-induced nephrotoxicity, but also the accelerated renal aging and eventual progression to CKD.

3116 Accumulation And Efflux Characteristics Of Diglycolic Acid In Human Proximal Tubule Cells

C. Robinson, E. Luttrell-Williams and K. McMartin. *Pharmacology, TOxicology & Neuroscience, LSU Health Sciences Center - Shreveport, Shreveport, LA.*

Diglycolic acid (DGA) has been shown to be the toxic metabolite of diethylene glycol (DEG). DGA elicits significant proximal tubule cell necrosis, which is the hallmark sign of DEG toxicity, eventually leading to acute kidney failure following ingestion. In previous studies, our laboratory has shown that DGA accumulates remarkably in kidney tissue, *in vivo*. Therefore, we hypothesize that DGA accumulates in human proximal tubule (HPT) cells *in vitro*, due to the disruption of uptake and efflux. To test this hypothesis, we analyzed the total uptake and time course efflux of DGA in HPT cells. Human kidney proximal tubule cells were cultured until confluent, then subcultured onto membrane inserts in 24 well plates, allowing for distinct apical and basolateral uptake/efflux. Cells were treated with 14C-DGA, apically and basolaterally, and allowed to incubate for 4 hours. Cells were then rinsed and fresh buffer was added. Cells were processed and uptake, as well as efflux, was measured from both apical and basolateral interfaces. HPT cells demonstrated more DGA uptake from the basolateral direction, compared to the apical. Moreover, there was virtually no efflux of DGA form either apical or basolateral direction, even at later time points. These results suggest a mechanism of increased DGA uptake from the basolateral direction, while mimicking the accumulation characteristics observed in our *in vivo* studies. These studies indicate that efforts to stimulate efflux or to inhibit uptake might have potential therapeutic implications.

3117 Establishment and Characterization of Kidney Drug Interaction Models by Stably Expressing hOAT1 in HEK and RPTEC Cell Lines

A. Briley and C. Zou. *ATCC, Gaithersburg, MD.* Sponsor: K. Donato.

In vivo studies have shown that kidney membrane transporters play a key part in drug disposition and renal clearance. One such transporter is OAT1 (SLC22A6) which is critical for maintaining homeostasis of endogenous substances. This makes OAT1 a good transporter to use to assay for drug interactions with the kidney. Unfortunately primary cells lose OAT1 expression in culture, and transiently expressed OAT1 has large or great variations between production lots which makes data hard to interpret. In our study we have generated HEK-293T/17 and RPTEC-TERT1 cells that stably overexpress the OAT1 gene driven by an EF1α promoter. After confirming the mRNA expression by RT-PCR, we performed immunostaining showing that OAT1 is correctly trafficked to the membrane. Most importantly, we validated that the overexpressed OAT1 transporter has normal transport activities by using 5-CF (5-Carboxyfluorescein) and PAH (Para-aminohipurate) uptake assays and the uptake can be inhibited by the well know inhibitors probenecid and novobiocin. Both inhibitors responded in a dose dependent manner for 5-CF uptake with IC50 values between 5-15uM. Even at higher passages, both cell lines retain the functionality of OAT1. Overall, our data has shown that these modified cell lines are very useful tools , which will provide improved consistency over time versus current models, for *in vitro* testing of how compounds are affected by the OAT1 membrane transporters.

3118 A Multiplexed Approach to Predict Kidney Toxicity *In Vitro* Using Heme Oxygenase-1 and Quantitative Phenotypic Readouts

S. Ramm[1,2], M. Adler[1,2] and V. S. Vaidya[1,2]. [1]*Department of Medicine, Renal Division, Brigham and Women's Hospital, Boston, MA and* [2]*Department of Systems Biology, Laboratory of Systems Pharmacology, Harvard Medical School, Boston, MA.*

Kidney toxicity due to drugs and environmental chemicals accounts for significant patient mortality and morbidity, and yet existing *in vitro* screening methods lack sufficient sensitivity and specificity to prevent these agents from reaching humans. We previously showed that cellular expression of heme oxygenase-1 (HO-1) serves as a robust and sensitive biomarker for *in vitro* nephrotoxicity screening using human proximal tubular epithelial cells (HPTECs) either cultured in 2-dimensional plates or in an *ex vivo* three-dimensional microphysiologic system (kidney on-

a-chip). The objective of this study was to build on our previous work and develop a novel predictive systems toxicology platform enabling measurement of HO-1, traditional toxicity endpoints (ATP, cell number, dead cells) along with quantitative and mechanistic high-content imaging based perturbations to mitochondria, ROS, endoplasmic reticulum (ER), and cell morphology. HPTECs were treated with a panel of 39 mechanistically distinct proximal tubule (PT) toxic compounds over 6 doses and at 4 time points. According to the area under the receiver-operator characteristic curve (AUROC) of individual parameters, HO-1 (0.87) and nuclear shape (0.87) performed better than traditional endpoints of cell viability, like ATP (0.73). However, the predictive ability could be further improved by combining HO-1 with cell count (0.92), ER intensity (0.92), mitochondrial intensity (0.93), or cell morphology profile (0.98) using logistic regression. Using this multiplexed readout of HO-1 and cell profile we were able to correctly classify 28 of 30 PT-toxic compounds (sensitivity = 93%) and 9 of 9 non-toxic controls (specificity = 100%). These results suggest that a multidimensional panel of biomarkers that combines HO-1 along with mechanistic cellular perturbations provides a rapid, sensitive and specific approach to screen nephrotoxic agents for drug development and chemical risk assessment.

PS 3119 *Ex Vivo* Induction of Polymyxin-Induced Nephrotoxicity in a 3D Microphysiological Model of a Human Kidney Proximal Tubule

E. J. Weber[4], M. Vaara[2], T. Vaara[2], T. Neumann[3], J. Himmelfarb[1] and E. Kelly[4]. *[1]Kidney Research Institute, Seattle, WA; [2]Northern Antibiotics Ltd., Helsinki, Finland; [3]Nortis Inc., Seattle, WA and [4]University of Washington, Seattle, WA.*

With increasing resistance to current antibiotics, there is an urgency to develop new antibiotics to combat this threat. In the field of antibiotic development, identification of new classes of drugs has stagnated, thus alternative strategies involve approaches to improve existing antibiotics. Polymyxin antibiotics are highly effective in treating gram-negative bacterial infections but toxicities (particularly nephrotoxicity) have limited their applications. Prediction of drug induced-nephrotoxicity continues to be a challenge due to poor *in vitro–in vivo* recapitulation from traditional models such as cell culture and animals. Therefore a dire need remains for more robust models that accurately portray human physiology while at the same time following the toxicological necessities to reduce, replace, and refine animal model use. We have recently developed a 3D microphysiological system (MPS) that replicates many functions of the human kidney proximal tubule (PT) using a microfluidic culture technique. The PT is the primary site of polymyxin nephropathy and the goal of this study was to assess the utility of the MPS for modeling this toxicity. In preliminary studies, we have found that exposure of the MPS to concentrations of polymyxin B (50 μM-48 h) that induced tubular injury *in vivo*, resulted in a significant induction of cell-associated heme oxygenase-1 (HO-1), a renal injury biomarker. Furthermore, effluent analysis for established urinary biomarkers revealed a significant upregulation of kidney injury molecule-1 (KIM-1) in response to polymyxin exposure. Currently, the MPS has demonstrated an increased sensitivity to drug-induced injury, relative to traditional 2D culture, making it a potential resource for not only understanding chronic toxicity but mechanisms of toxicity as well. Thus ongoing studies will include RNA-seq analysis to identify the unclear mechanism(s) of polymyxin-induced nephrotoxicity. Furthermore, we hope to apply this knowledge when testing novel polymyxin analogs currently under development for improved clinical safety profiles.

PS 3120 Compensatory Hypertrophy and the Transport of Inorganic Mercury in Isolated Perfused Proximal Tubules

C. Bridges[2], D. W. Barfuss[1], L. Joshee[2] and R. K. Zalups[2]. *[1]Department of Biology, Georgia State University, Atlanta, GA and [2]Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA.*

Chronic kidney disease (CKD) is characterized by a progressive and permanent loss of functioning nephrons. In order to compensate for this loss, the remaining functional nephrons undergo glomerular and tubular changes. We hypothesized that these compensatory changes may alter the exposure and susceptibility of individual nephrons to nephrotoxicants, such as mercury. To test this hypothesis we compared the transport of mercuric species in individually perfused proximal tubules isolated from normal (Control) or uninephrectomized (NPX) rabbits. Kidneys of NPX rabbits experienced significant hypertrophy during the three weeks following surgery. Tubules isolated from NPX rabbits were significantly larger in diameter than those from Control rabbits. When tubules from Sham and NPX rabbits were perfused with cysteine-S-conjugates of inorganic mercury (Hg2+), we found that the luminal disappearance flux and tubular accumulation of Hg2+ was greater in tubules from NPX animals. This increase was inhibited by the addition of various amino acids. Moreover, RT-PCR analyses indicated that the expression of selected membrane transporters was greater in kidneys of NPX animals than in Control animals. Taken together, our data suggest that hypertrophic changes in proximal tubules may lead to an increased ability and/or tendency of proximal tubules to take up and accumulate nephrotoxicants such as Hg2+.

PS 3121 Evaluation of Compound Toxicity in Characterized Human Renal Proximal Tubule Epithelial Cells

S. Li. *NCATS,NIH, Rockville, MD.*

Kidney toxicity is a major problem in both drug development and the clinical setting. It is difficult to predict nephrotoxicity due to a lack of appropriate *in vitro* cell models, limited number of endpoints, and poor expression of membrane transporters in currently available kidney cell lines. We recently developed a pseudo-immortalized proximal tubule cell line (SA7K) from human primary renal proximal tubule epithelial cells. This cell line was characterized by the presence of proximal tubule cell-specific markers as well as several functional properties. In addition, transporter mRNA expression was profiled and cellular response to a limited number of known human nephrotoxicants was measured. Based upon positive early results, a larger study was designed to evaluate the response of SA7K cells to over 70 nephrotoxic and negative compounds. The human immortalized kidney cell line HK-2 was used as a comparator cell line. Three endpoints (cell viability, cell apoptosis and mitochondrial membrane potential) were assessed and the assays were performed on a quantitative high throughput screening platform (qHTS). Among over 40 nephrotoxic compounds, approximately 70% of the compounds were positive in at least one of these assays. There were notable differences in sensitivity to several drugs between HK-2 and SA7K cells (e.g. cyclosporin A), which may be due to differences in transporter expression. Our multiplexed qHTS platform using SA7K cells may be promising for early detection of renal toxicants.

PS 3122 Effects of Cadmium on Zinc, Copper and Other Essential Metals in Renal Cortex

W. C. Prozialeck, P. C. Lamar and J. R. Edwards. *Pharmacology, Midwestern University, Downers Grove, IL.*

Cadmium is a nephrotoxic pollutant that causes generalized proximal tubule dysfunction. These effects may result from the ability of Cd to alter the levels and function of metals such as Zn, Cu and Fe within the kidney, although the specific mechanisms are unknown. To further examine this possibility, we examined the effects of subchronic Cd exposure on tissue levels of a panel of metals (Ca, Cu, Fe, Zn, K, Mg, Mn) in the rat renal cortex. Adult male Sprague-Dawley rats were treated with CdCl2 (0.6 mg Cd/kg body weight in isotonic saline by subcutaneous injection, 5 days per week for 6, 9 or 12 weeks). At each time point, 24 hour urine samples were collected and assayed for levels of protein, creatinine, Kim-1, β2 microglobulin and cystatin C. Samples of renal cortex (n=3 per treatment group) were harvested and assayed for levels of the metals of interest by inductively-coupled mass spectrometry at Michigan State University. Data were analyzed by two-way ANOVA with Tukey's post hoc test. Results showed that at 9 and 12 weeks, Cd caused a significant increase in urine volume and urinary protein with no change in creatinine excretion. Increases in the excretion of β2 microglobulin, cystatin C and Kim-1 were evident as early as 6 weeks. Results of the metal analyses showed that Cd caused significant increases (~ 100-120%) in tissue levels of Cu at all of the time points examined. Tissue levels of Zn were transiently elevated at 6 weeks (control= 95±2 ppm (dry weight), Cd-treated=150±5 ppm), but declined to control levels at 9 and 12 weeks. Cd had no significant effects on any of the other metals, although there was a trend toward reduced levels of Fe in samples from Cd-treated animals that did not reach statistical significance. Tissue levels of Cd were 530±52, 863±36, 837±23 ppm dry weight at 6, 9 and 12 weeks respectively. These results indicate that the early stages of Cd nephrotoxicity are associated with alterations in renal tissue levels of Zn and Cu and they suggest possible roles for these metals in the pathophysiology of Cd-induced kidney injury.

3123 Early, Sensitive and Mechanistic Detection of Drug-Induced Kidney Injury in Humans Using Urinary KIM-1, miR-21, -200c and -423

M. Pavkovic[5], A. S. Chua[1], V. Bijol[1], O. Nicoara[1], M. Cárdenas-González[5], K. Ramachandran[1], J. Himmelfarb[2], S. S. Waikar[1] and V. S. Vaidya[1,5]. *[1]Department of Medicine, Brigham and Women's Hospital, Boston, MA; [2]Department of Medicine, University of Washington, Seattle, WA; [3]Department of Neurology, Brigham and Women's Hospital, Boston, MA; [4]Department of Pathology, Brigham and Women's Hospital, Boston, MA and [5]Laboratory of Systems Pharmacology, Harvard Medical School, Boston, MA.*

Up to 1/3 of hospitalized acute kidney injury (AKI) patients can be attributed to drug toxicity. Additionally, nephrotoxicity is also an issue during drug development. Clinical diagnosis of AKI still utilizes serum creatinine (SCr) acknowledged as insensitive and nonspecific. In an attempt to identify biomarkers (BMs) for improved AKI diagnosis, we investigated a multidimensional approach that included Kidney Injury Molecule-1 (KIM-1) and candidate miRNAs (miR-21, -200c and -423) in a longitudinal cohort of mesothelioma patients (n=108) receiving cisplatin (Cp) therapy. All BMs were increased after treatment, also in patients without clinical AKI diagnosis. The three miRNAs were significantly increased ($p<0.01$) as early as 4h after Cp administration although >90% of the patients were not diagnosed with AKI based on SCr levels until 12h. Using *in situ* hybridization we detected an increased expression of miR-21 in injured tubules in biopsy sections from AKI patients, suggesting the kidney to be the likely source of this miRNA in urine. Complementary, *in vitro* experiments mimicked *in vivo* findings with significantly increased (1.7-3.1 fold; $p<0.001$) levels of all three miRNAs in the medium of primary human proximal epithelial cells after a 24h treatment with Cp. Furthermore, target analysis supports the potential of miRNA profiles in urine to reflect pathological processes in the kidney. In summary, we provide evidence for the value of using more the one BM and thus combining the sensitivity of KIM-1 along with early diagnostic and mechanistic potentials of candidate miRNAs for non-invasive detection of drug-induced AKI in humans.

3124 Inhibition of Cyclosporine A (CsA)-Induced Renal Dysfunction and Fibrosis by Chrysin and Apigenin

R. Nagavally and S. M. Ford. *Toxicology Program, St. John's University, Jamaica, NY.*

CsA is the drug of choice for immunosuppression in organ transplantation. A major drawback of CsA is nephrotoxicity including fibrosis. One mechanism contributing to fibrosis due to nephrotoxicants such as CsA is epithelial mesenchymal transition (EMT). EMT involves a shift from epithelial to mesenchymal phenotype subsequent to tissue injury. EMT occurs via loss of epithelial markers such as E-cadherin and acquisition of mesenchymal markers such as α-smooth muscle actin (α-SMA), and vimentin, resulting in increased cellular motility. We have previously shown that chrysin inhibits TGF-β1-induced EMT-associated behavior in LLC-PK1 cells by modulation of Akt signaling. This suggests a possible protective effect of chrysin on CsA-induced nephrotoxicity. Male Sprague Dawley rats were treated with CsA (25 mg/kg S.C) alone or with the flavonoids chrysin (50 or 10 mg/kg I.P) or apigenin (10 mg/kg I.P) for 28 days. Treatment with CsA caused a 20% increase in BUN at 28 days of treatment, 30% and 50% increase in serum creatinine at 14 and 28 days of treatment respectively and 15% and 20% decrease in creatinine clearance at 14 and 28 days of treatment respectively when compared to the controls. Co treatment with chrysin and apigenin abolished the changes caused by CsA. Histological examination showed that the flavonoids inhibited CsA-induced dilation and atrophy of tubules, cast formation within the tubules and mononuclear cell infiltration. The flavonoids inhibited fibrosis caused by CsA as evaluated by histological and quantitative Sirius red assays. Finally, treatment with the flavonoids also inhibited the expression of α-SMA induced by CsA. The protection offered by the flavonoids against CsA-induced nephropathy and the expression of mesenchymal markers may be related to its ability to inhibit TGF-β1-induced EMT in LLC-PK1 cells.

3125 Zn Prevent the Obesity Induced Kidney Dysfunction by Downregulating P38 MAPK Pathway

M. Luo[1,2], P. Luo[1], L. Miao[1] and L. Cai[2]. *[1]Nephrology, The Second Hospital of Jilin University, Changchun, China and [2]Pediatrics, University of Louisville, Louisville, KY.* Sponsor: L. Cai.

Objective: Obesity, a common public health challenge, is considered a secondary factor that raises the risk of renal diseases especially in youth. Zinc (Zn) is essential for multiple organs. However, its effects on the kidneys of obese individuals, particularly at a young age, have not been well studied. Methods: 4 week old C57BL/6J male mice were fed normal diet (ND) or high fat diet (HFD) containing one of three levels of Zn (30, 60, 90 mg/4057 kcal, named ZL, ZN and ZH, respectively) for 3 months. To define the role of P38 MAPK in HFD-induced renal damage, age matched C57BL/6J male mice were fed with HFD/ZN and HFD/ZL with and without SB203580, a P38 MAPK inhibitor, for 3 months. Results: Significant HFD-induced obesity accompanied by kidney hypertrophy, mesangial matrix accumulation, and proteinuria was observed along with increased renal inflammation responses as reflected by upregulated p-P38 MAPK and inflammatory markers: MCP-1, IL-1β, IL-6 and TNF-α. Renal effects of HFD-induced obesity worsened in HFD-ZL group but were prevented almost completely in HFD-ZH group. SB203580 blocked P38 MAPK activation and also prevented HFD-induced, and Zn deficiency-worsened, renal dysfunction and inflammation. Conclusion: These results indicated that renal inflammatory responses played a central role in the pathogenesis of HFD-induced renal dysfunctions. Zn deficiency and supplementation could aggravate and prevent, respectively, disease progression mainly through modulating P38 MAPK. This study reveals a potential for Zn application in the early prevention of obesity-related glomerulopathy, particularly in youth.

3126 Examination of the Protective Effect of Resveratrol on Mitochondrial Function and Oxidative Stress Following Exposure of HK-2 Cells to Cisplatin

M. Valentovic, J. G. Ball, R. Murphy, A. B. Lamyaithong and A. Schnelle. *Pharmacology, Marshall University School of Medicine, Huntington, WV.*

The cancer chemotherapeutic agent cisplatin is used in treating many cancers including testicular, ovarian and cervical cancer. Impaired renal function is a major side effect of cisplatin and occurs in 33% of patients treated with cisplatin. Resveratrol (RES) is a polyphenolic compound that possesses anticancer activity. Part of the mechanism for reducing cisplatin toxicity by RES may be mediated by maintaining mitochondrial function and reducing oxidative stress. The purpose of this study was to evaluate the effect of cisplatin on mitochondrial function in a human proximal tubular kidney cell line (HK-2). Specifically the levels of ATP and ADP were measured following exposure to cisplatin in the presence of RES or DMSO (vehicle). HK-2 cells were pretreated for 1 h with 0-15 uM RES (vehicle control 10% DMSO). Renal cells were subsequently exposed to cisplatin at a final concentration of 0-30 uM for 24 or 48 h. Viability was assessed using MTT release and cell count with an n=6/treatment group and repeated 3 separate times. Cells pretreated with 10 uM RES were protected from cisplatin cytotoxicity at 24 and 48 h. Cisplatin caused a concentration dependent decline in MTT viability after 24 h exposure to 0, 7.5 and 15 uM cisplatin. RES pretreatment for 1 h with 10 or 15 uM RES increased HK-2 viability relative to cells exposed only to RES Vehicle (DMSO). Additional studies examined whether RES stimulated cell growth as part of the mechanism for cellular protection. RES did not increase cell number as part of its protective mechanism. ATP levels were diminished by cisplatin causing an increase in the relative balance of ADP/ATP. RES (5 and 7.5 uM) did not alter ATP or ADP/ATP ratios. Additional studies examined Caspase cleavage, protein carbonylation and 4-hydroxynonenal (4-HNE) adduction following cisplatin exposure of HK-2 cells pretreated with DMSO or RES. Western analysis detected an increase in protein carbonylation with 24 h cisplatin exposure which was reversed by RES. In summary, RES protects human proximal tubular epithelial cells from cisplatin cytotoxicity and preserves mitochondrial ATP levels and does not increase cell number during the treatment period. (Supported by NIH Grants INBRE 3P20RR016477-09S4; 5P20RR016477 and 8P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence).

3127 Cadherin Expression, Vectorial Active Transport, and Metallothionein Isoform 3 Mediated EMT/MET Responses in Cultured Primary and Immortalized Human Proximal Tubule Cells

A. Slusser, C. S. Bathula, D. A. Sens, S. Somji, M. A. Sens, X. D. Zhou and S. H. Garrett. *Pathology, University of North Dakota, Grand Forks, ND.*

The proximal tubule of the kidney is particularly susceptible to toxicant-induced damage and cell cultures of human proximal tubule cells are widely utilized to study the role of epithelial-mesenchymal transition (EMT) in renal disease. Cadmium is a heavy metal that is known to produce renal tubular necrosis, and accumulates in the proximal tubule. This metal binds to a family of cysteine rich metal binding proteins known as metallothioneins (MT) that are found in abundance in the kidney. Previous studies from our laboratory have shown that the third isoform of metallothionein (MT-3) is expressed in the epithelial cells of the human kidney, including the cells of the proximal tubule. An immortalized proximal tubule cell line does not express MT-3 and does not demonstrate vectorial active transport. Transfection of the MT-3 gene into the HK-2 cells restores vectorial active transport as evidenced by dome formation. This suggests that MT-3 is involved in mesenchymal to epithelial transition (MET). The goal of this study was to define the role of growth media composition on classic EMT responses, define the expression of E- and N-cadherin, and define the functional epitope of MT-3 that mediates MET in HK-2 cells. It was shown that both E- and N-cadherin mRNA and protein are expressed in the human renal proximal tubule. Based on the pattern of cadherin expression, vectorial active transport, and transepithelial resistance, it seems that the HK-2 cell line has already undergone many of the early features associated with EMT. Our data also shows that the unique, six amino acid C-terminal sequence of MT-3 is required to induce MET in HK-2 cells. Thus the HK-2 cell line can be an effective model to study later stages in the conversion of the renal epithelial cell to a mesenchymal cell and when transfected with MT-3 it may be an effective model to study the process of MET.

3128 Mechanistic Role of Phospholipase D4 in Regulating Kidney Fibrosis

P. Trivedi[2], R. Kalyan[2], C. Huber[1], D. Nemazee[1] and V. Vaidya[2]. [1]*The Scripps Research Institute, La Jolla, CA and* [2]*Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Kidney fibrosis is the hallmark of chronic kidney disease leading to end-stage renal failure. In order to map the molecular pathogenesis of kidney fibrosis, we performed RNA sequencing and identified Phospholipase D4 (PLD4) as one of the highly upregulated genes in mouse model of folic acid (FA)-induced nephropathy. The mRNA levels and *de novo* protein synthesis of PLD4 increased ~ 20 fold in three mechanistically distinct mouse models as well as in patients with biopsy-proven kidney fibrosis. To investigate the functional role of PLD4, we subjected PLD4 knockout (PLD4-/-) and wild type (PLD4+/+) mice to unilateral ureteral obstruction (UUO)-induced kidney fibrosis. PLD4-/- mice showed significantly less ($p<0.05$) fibrosis compared to the PLD4+/+ mice at day 10 following UUO as revealed by the protein expression of collagen1α and TGF-β signaling molecules pSmad2 and pSmad3. Increased TGF-β signaling in PLD4+/+ mice was associated with significantly ($p<0.001$) increased M2 macrophages (Arg1 expression using qPCR). Surprisingly, flow cytometric analysis revealed significantly increased CD8 cells in the kidney of PLD4-/- compared to the PLD4+/+ mice at baseline and following UUO ($p<0.01$). Pro-inflammatory cytokines (IL-2, IL-6, IL-1β, IL-17 gene expression) were high in the PLD4-/- compared to the PLD4+/+ mice following UUO. Mechanistically, using whole genome RNA sequencing, we show significantly reduced ($p<0.001$) expression of serpina1d gene at baseline, which translates to alpha-1 antitrypsin (AAT), in the PLD4-/- compared to the PLD4+/+ mice. In the PLD4+/+ mice, AAT levels decreased following fibrosis to allow protease activation and degradation of collagen. Whereas, sustained activation of the proteases due to absence of AAT in PLD4-/- mice led to an efficient degradation of collagen rescuing these mice from scar tissue formation in the kidney. Thus targeting PLD4 has the potential to be a novel therapeutic strategy for kidney fibrosis.

3129 Investigating the Mechanistic Basis of Mitochondrial Dysfunction in Adefovir and Tenofovir Cytotoxicity in Novel Human Proximal Tubule Cell Models

A. Tort, A. E. Chadwick, P. Murray, B. K. Park and D. J. Antoine. *Cellular and Molecular Physiology, University of Liverpool, Liverpool, United Kingdom; Pharmacology and Therapeutics, University of Liverpool, Liverpool, United Kingdom.*

Introduction: Mitochondria are known to be a target for a broad spectrum of renal toxins. Nonetheless, detection of mitochondrial impairment due to drug exposure becomes a challenging task using *in vitro* cultures due to the Crabtree effect and a lack of physiologically relevant *in vitro* renal models. Our aim was to explore the role of mitochondrial perturbation on the mechanism of renal toxicity induced by anti-retrovirals by driving ciPTECs (human conditionally immortalized proximal tubule epithelial cells) to a higher aerobic metabolic state to develop an *in vitro* model that has the potential to screen for mitochondrial nephrotoxicity in drug development. Methods: CellTiter-Glo luminescent cell viability assay and lactate dehydrogenase were performed on ciPTECs following adefovir and tenofovir treatments. Cells were cultured and dosed in glucose versus galactose media. Seahorse technology was used to measure ciPTECs bioenergetic metabolism and mitochondrial function. Results and Discussion: Cells cultured in galactose conditions showed a significant upregulation of mitochondrial oxidative phosphorylation (OXPHOS) compared to glucose conditions, which resulted in a higher susceptibility towards mitotoxins (Rotenone EC50ATPglu / EC50ATPgal > 3125, $p < 0.0001$). When treated with adefovir, cytotoxicity was observed in both glucose and galactose conditions, yet the EC50 in galactose cultured cells was much lower (EC50 Gluc= 15.3 μM vs. EC50 Gal= 2.5 μM). These results point out that one of the potential toxicity targets of adefovir is the mitochondria. Tenofovir treatment resulted in a significant increase in extracellular acidification after 15 days, which could be an initial sign of mitochondrial impairment. Conclusions: Galactose based medium confers a significantly different bioenergetic configuration to ciPTECs that increases their susceptibility to clinically relevant renal mitotoxins. Thus, galactose cultured ciPTECs have the potential to be an advantageous model to identify mitochondrial toxicity.

3130 Urinary Micrornas Response in Adults Exposed Chronically to Fluoride in Drinking Water

S. Solis-Angeles[3], M. d. C. González-Horta[1], M. I. Jimenez-Córdova[3], M. d. C. Cárdenas-Gonzalez[2], E. E. Villarreal-Vega[1], G. Aguilar-Madrid[4], L. M. Del Razo[3] and O. C. Barbier[3]. [1]*Facultad de Química, Universidad Autonoma de Chihuahua, Chihuahua, Mexico;* [2]*HMS Systems Biology WAB 536, Harvard Medical School, Boston, MA;* [3]*Toxicología, Cinvestav-IPN, Mexico DF, Mexico and* [4]*Unidad de Investigación en Salud en el Trabajo, IMSS, Mexico DF, Mexico.*
Sponsor: O. Barbier.

Fluoride in drinking water exceeds safe limits for human consumption in various regions of the world. An example is the northern region of Mexico, where the underground soil is rich in fluorine minerals and where drinking water frequently exceeds the safety limits of fluoride. In addition, the incidence of dental fluorosis by chronic exposure to fluoride is high. Kidney is the major excretory organ of fluoride, through the urine. Proximal convoluted tubule is the main segment of the nephron involved in fluoride reabsorption and chronic exposure can affect the redox status and the tubule structure. The main effects associated with early damage to the proximal convoluted tubule are epithelial-to-mesenchymal- transition, hypoxia, apoptosis and inflammation; all these processes are accompanied by changes in gene expression. Urinary miRNAs due to their structural stability and tissue-specificity emerge as potential markers for understanding the mechanisms by which renal injury occurs at early stages. In previous studies, we have investigated an increase on the concentration of proteins markers for early kidney damage in a adult population (n=239) of northern Mexico exposed to high levels of fluoride (>2 mg/L) in drinking water. In this study we aimed to identify changes in the expression profile of a set of 45 miRNAs associated with early kidney damage, like miR-21, miR-200 family, miR-192, miR-205 and miR-194. A selection of 48 male participants from 239 (24 high exposed and 24 low exposed to fluoride) for total RNA extractionand MiRNA evaluation using qRT-PCR technique. Changes in their expression, more than 1.3-fold, for several miRNAs were predicted to be target genes of miRNAs through TargetScan analysis, due to fluoride exposure. Partially supported by Thematic Network Collaborative, PROMEP-SEP and Conacyt 239689.

3131 Nox-2 Mediated Mir21 Promotes Mesangial Cell Activation and Kidney Inflammation in Environmental Toxin Potentiation of Nonalcoholic Fatty Liver Disease

F. Alhasson. *Environmental Health Science, University of South Carolina, Columbia, SC.* Sponsor: S. Chatterjee.

We have shown previously that CYP2E1-mediated oxidative stress and circulating damage associated molecular patterns 9DAMPS) in nonalcoholic fatty liver disease (NAFLD) cause mesangial cell-induced tubular inflammation. Extending the studies, we hypothesized that mesangial cell activation was dependent on NOX-2 mediated mir21 increase in the kidneys of NAFLD mice. The study used bromodichloromethane, a disinfection byproduct of drinking water as a second hit in causing potentiation of NAFLD in mice. High fat diet (60%kcal) was used to induce fatty liver phenotype with parallel insulin and leptin resistance. Kidneys were collected after 4 weeks of toxin exposure and probed for mesangial cell activation and tubular inflammation. Results showed that NAFLD kidneys had higher 3-nitrotyrosine, a marker of peroxynitrite formation and proinflammatory mediators IL-1b and TNF-a. The kidneys also had significant increases in a-SMA, a marker of mesangial cell activation. Since we have shown previously a role of NADPH oxidase in NAFLD progression, we used p47 phox deficient mice to probe the role of peroxynitrite formation and mesangial cell activation.. p47 phox deficient mice had significantly decreased 3-nitrotyrosine levels and a-SMA immunoreactivity suggesting the direct role of NOX-2 in mesangial cell activation. Peroxynitrite signaling has been shown by us and others to activate proinflammatory pathways. To show that peroxynitrite generation could act through mir21-induced inflammatory pathways, we used mir21 knockout mice. Results showed that mir21 knockout mice had significantly decreased mesangial cell activation and inflammation but was TLR4 independent since TLR4 knockout mice failed to show decreased mesangial cell activation. Finally both p47phox and mir21 knockout mice showed decreased tubular immunotoxicity and proinflammatory mediator release. In conclusion we show that NOX-2 plays a significant role in kidney inflammation following progressive NAFLD. The above process might involve mir21 dependent mesangial cell activation. Fund: R00ES019875 to SC

3132 Effect of Cadmium on the Expression of Stem Cell Markers in Human Proximal Tubular Cells

S. Shrestha, S. H. Garrett, D. A. Sens and S. Somji. *Pathology, University of North Dakota, Grand Forks, ND.*

The proximal tubules of the kidney are a common site of toxic insult, cell death and regeneration as well as a major site for the development of renal tubular diseases. Toxic insult can result from heavy metals, pharmaceuticals, diabetes induced nephropathy or from ischemia. In this study, we characterized stem cell populations within mortal cultures of human proximal tubular cells (HPT) and the immortalized cell line HK-2 as well as in spheroids isolated from both the cultures. Four stem cell markers were chosen, two of which have been shown to be expressed in renal stem cells *in vivo*, CD24 and CD133, as well as CD44 and ALDH1A1 which are commonly expressed in many other stem cell niches. Mortal cultures of human proximal tubule cells as well as the spheroids derived from them expressed high levels of ALDH1A1, CD24, CD44 and CD133 whereas the expression of these markers in the HK-2 cells was decreased when compared to the HPT cells. Acute exposure of HPT cells to 4.5µM, 9µM or 27µM of cadmium for 24 h resulted in an increase in ALDH1A1 mRNA while no significant changes were seen in the expression levels of the other genes. Chronic exposure of HPT cells to 4.5µM, 9µM or 27µM of cadmium for 13 days resulted in a decrease in CD133 mRNA while the expression levels of other three markers were unchanged. HPT cells were also continuously passaged three times in 0.5µM, 1µM or 2 µM cadmium or 7.5, 11 or 16 mM glucose and the expression levels of stem cell markers was assessed. The data obtained showed that continuous passage in low doses of cadmium resulted in a decrease in the expression of stem cell markers whereas exposure to high levels of glucose did not have an effect on the expression of these markers. In conclusion, our results suggest that exposure to cadmium may alter the population of stem cells in the proximal tubules of the kidney and these cells could potentially be involved in the regeneration process after tubular insult.

3133 M2 Macrophages May Be Involved in the Aggravation of Renal Tubulointerstitial Fibrosis in Rats with Unilateral Ureteral Obstruction Caused by Fluoride Exposure

T. Kido[3], M. Tsunoda[1], C. Sugaya[1], H. Hano[3] and H. Yanagisawa[3]. *[1]Hygiene, Kitasato University School of Medicine, Kanagawa, Japan; [2]Pathology, The Jikei University School of Medicine, Tokyo, Japan and [3]Public Health and Environmental Medicine, The Jikei University School of Medicine, Tokyo, Japan.*

The contamination of ground water by fluoride (F) has been reported in China and India. Since most of F is filtered by the kidney (approximately 70%), humans or experimental animals with renal damage may be affected by F exposure more seriously than those with normal kidney function. We explored whether or not F exposure aggravated tubulointerstitial fibrosis seen in rats with UUO. It has been recently documented that macrophages promote the production of extracellular matrix and migration of fibroblast. Based upon the background, we examined the effects of F exposure on the aggravation of tubulointerstitial fibrosis with relation to macrophages (M1 and M2) and α-SMA in the UUO setting. Left ureter of 6-week-old male rats was ligated by 5-0 silk (obstructed kidney: OK). F was administered to rats with UUO at 0, 75 and 150 ppm in the drinking water for 2 weeks. After 2 weeks, the kidney sections were immunohistochemically stained with anti-ED-1, anti-ED-2, anti-ED-3 and anti-α-SMA antibodies to examine the macrophage subtypes, ED-1 (immature macrophages), ED-2 (M2 macrophages), ED-3 (M1 macrophages) and the fibroblast marker, α-SMA. The α-SMA positive area was determined by imaging analysis and the ED-1, ED-2 and ED-3 positive cell number was counted within the renal cortex. The mean values of the α-SMA positive area and the ED-1, ED-2 and ED-3 positive cell number were significantly increased in the OK from 150 ppm F exposure when compared to the OK from the controls (0 ppm F exposure). The significant correlations between the number of ED-2 positive cells and the α-SMA positive area were observed in the OK from UUO rats exposed to each dose of F using the Spearman rank-order test. It is indicated that F exposure develops the tubulointerstitial fibrosis formation seen in rats with UUO via increased M2 macrophage invasion.

3134 Detection of Kidney Injury in Mexican Children Exposed to Environmental Toxicants

M. Cardenas Gonzalez[6], I. Perez Maldonado[2], O. Barbier[4], O. Gaspar[3], M. Medeiros[5], V. Sabbisetti[1] and V. Vaidya[1,6]. *[1]Brigham and Women's Hospital, Boston, MA; [2]CIACYT, San Luis Potosi, Mexico; [3]CIATEJ, Nuevo Leon, Mexico; [4]CINVESTAV-IPN, Mexico DF, Mexico; [5]Hospital Infantil Federico Gomez, Mexico DF, Mexico and [6]Laboratory of Systems Pharmacology, Harvard Medical School, Boston, MA.*

Mexican population has a high and concerning risk for initiation and progression of chronic kidney disease (CKD). The exposure to environmental kidney toxicants together with comorbid conditions, are well recognized as risk factors for CKD. Children represent the most susceptible population to kidney toxicants, due to their unique routes of exposure and their special vulnerabilities. The two objectives of this study were 1) to measure the levels of arsenic (As), cadmium (Cd), chromium (Cr) and fluoride (F) in children living in semi-urban and urban areas of Mexico and 2) to associate the levels of exposure with the levels of kidney injury biomarkers: kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL). In a cross sectional study we assessed children (6-11 years) living in Villa de Reyes SLP (semi-urban, n=83) or Mexico City (urban, n=100). Exposure to As and F was higher in semi-urban group ($p<0.005$), whereas Cr was higher in urban group ($p=0.005$). Overall in both groups As and Cr levels were exceedingly high (7 and 1.5 -times higher, respectively than the occupational Biological Exposure Index). Although the kidney function evaluated by routine clinical tests was normal, urinary KIM-1 and NGAL were high in both groups. KIM-1 was similar in both groups whereas, NGAL was higher in the semi-urban ($p<0.005$). Using adjusted regression model, we found that higher levels of Cr were significantly associated with higher levels of urinary KIM-1 in the semib-urban group ($p=0.01$). There was no significant association between As, Cr or F exposure and the levels of KIM-1 or NGAL in urban group. In summary, we report that children living in semi-urban and urban regions of Mexico are exposed to significantly high levels of kidney toxicants that have the potential to cause kidney tubular injury as indicated by urinary biomarkers.

3135 Bromate-Induced Alterations in the Expression of Cyclin-Dependent Kinase Inhibitors via Epigenetic Mechanisms

R. Kolli, B. Cummings and T. Glenn. *Environmental Health Science, University of Georgia, Athens, GA; Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA.*

Bromate (BrO3-) is a drinking water disinfection byproduct formed from naturally occurring bromide during the ozonation process. Our laboratory previously showed that BrO3--induced nephrotoxicity *in vitro* and *in vivo* correlated to increased expression of the cyclin-dependent kinase (CDK) inhibitor p21. We also showed that the DNA methyltransferase inhibitor 5-aza-2-deoxycytidine and the histone deacetylase inhibitor trichostatin A increased the expression of p21 indicating the role of both DNA methylation and histone acetylation in regulation of the p21 expression. We used next-generation sequencing to demonstrate that the promoter region of the p21 gene in untreated human embryonic kidney (HEK293) cells displays low level of methylation (0.8%). Treatment of the cells with BrO3- failed to significantly alter this value, suggesting that promoter demethylation at the CpG islands spanning 500bp upstream of and including the transcription start site is not a major mechanism by which BrO3-increased p21 expression in kidney cells. In contrast, exposure of normal rat kidney (NRK) cells to similar concentrations of BrO3- altered histone acetylation at the promoter region spanning 200bp upstream from the start site, as determined using chromatin immunoprecipitation (ChIP) assays, in a concentration- and time-dependent manner that correlated to changes in p21 protein expression. The data suggest the novel finding that BrO3- regulates p21 expression in kidney cells using mechanisms that alter histone acetylation, and not by inducing de-methylation of the p21 gene, especially at promoter region spanning the transcription start site.

3136 Usefulness of Adult Zebrafish for Screening Drug-Induced Kidney Injury: Histopathological Assessment

Y. Kato, Y. Tonomura, H. Hanafusa, K. Nishimura, C. Kondo, S. Matsushima, T. Fukushima, M. Kaneto and M. Ueno. *Drug Safety Evaluation, Shionogi&CO.,LTD., Osaka, Japan.*

Drug-induced kidney injury (DIKI) is a profound safety issue in drug development. Although various efforts have been made to establish an *in vitro* system for DIKI, this system cannot fully reflect structures and functions *in vivo*. We herein focused on adult zebrafish as a small *in vivo* system, and pathologically evaluated its usefulness for screening DIKI. To explore renal pathological lesions, representative nephrotoxicants such as gentamicin (100 mg/kg, single) and doxorubicin (40 mg/kg/day for 4 days) were intraperitoneally administrated to adult zebrafish (Zebra Rerio). In the gentamicin experiment, zebrafish were sacrificed 24, 48, 72, and 96 hours after being treated. Renal tubular necrosis accompanied by increases in lysosomal bodies and degenerative mitochondria was observed by electron microscopy 24 hours after dosing. A regenerative epithelium was observed after 72 hours by light microscopy, and most renal tubules recovered by 96 hours. In the doxorubicin experiment, zebrafish were sacrificed 96 hours after being treated. The mild fusion of epithelial cell foot processes was observed ultrastructurally in the glomerulus. These tubular and glomerular changes were similar to those in the rat. To determine the accuracy of a diagnosis of nephrotoxicity, adult zebrafish were intraperitoneally treated with nephrotoxicants or non-nephrotoxicants for ~4 days, and then exsanguinated under tricaine anesthesia 24 hours after the final dosing. They were fixed in Davidson's solution and whole body sections were stained with hematoxylin and eosin. Of the 43 known nephrotoxic compounds, 28 cause DIKI in mammals, including gentamicin, cisplatin, doxorubicin, and β-lactam antibiotics. DIKI was judged by the occurrence and frequency of degenerative renal changes from histopathological control reference data (n=220). Thirteen out of 28 compounds caused DIKI in zebrafish (a true positive rate of 46%), with no false positives in non-nephrotoxicant-treated zebrafish (a true negative rate of 100%). These results suggested that the application of adult zebrafish to evaluations of DIKI using pathological examinations is useful because a screening system is available with small amounts of compounds and provides similar results to those in mammals without false positives.

3137 Inhalation Exposure to Traffic-Generated Air Pollutants Increases Renal Oxidative Stress, Matrix Metalloproteinase-9 Expression, and Fibrosis, Which are Mediated Through an Angiotensin II-Dependent Pathway

L. Schneider[2], J. Lucero[2], J. D. McDonald[1] and A. Lund[2]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM and* [2]*Biological Sciences, University of North Texas, Denton, TX.*

Multiple studies have revealed a clear correlation between exposure to traffic-generated air pollution and exacerbation of cardiovascular disease (CVD); however, very limited information exists on the effects of inhaled pollutants on the kidneys, even though chronic kidney disease (CKD) is an important risk factor for CVD. Dysregulation of the renin-angiotensin (Ang) system (RAS) is known to mediate atherogenesis and renal pathologies through mechanisms that involve upregulation of reactive oxygen species (ROS), matrix metalloproteinases (MMPs), and fibrosis, in addition to others. Thus, we hypothesize that MVE-exposure results in altered RAS signaling and subsequent induction of Ang II-mediated signaling pathways. To test this hypothesis, 10 wk old male Apolipoprotein (ApoE)-/- mice, on a high fat diet, received either the ACE inhibitor, captopril (ACEI: 4 mg/kg/day), or nothing (control) in their drinking water. Mice were then randomly assigned to inhalational exposure of either filtered-air (FA: n=10 FA+ACEI; n=10 FA+water) or a mixture of 150µg PM/m3 diesel exhaust + 50µg PM/m3 gasoline exhaust (MVE: n=10 MVE+ACEI; n=10 MVE+water) for 6 hr/day for 30 days. We observed a 2.1-fold increase in plasma Ang II levels, in addition to 2.9 -fold increase in expression of the angiotensin II receptor type 1 (AT1), in MVE-exposed mice compared to controls. MVE-exposure resulted in significant elevations in oxidative stress (TBARS) in the kidneys (17.7 ± 1.4 nmol/ml/mg), compared to FA (7.8 ±1.1 nmol/ml/mg), which was confirmed with DHE staining. MVE-exposed mice also showed increased renal MMP-9 expression (1.8-fold increase by immunofluorescence; in both nephron and tubules), which was associated with exacerbated renal fibrosis (scored using Masson's Trichrome). Treatment with an ACEI decreased TBARS and MMP-9 expression, and while fibrosis was also attenuated, it was still elevated above that observed in FA controls. Such findings indicate that inhalation exposure to traffic-generated pollutants can promote induction of factors associated with CKD, which are mediated in part through an Ang-II pathway. Funded by NIH R00ES0126586 and UNT RIG Grant GA9306 (AKL).

3138 Application of Small RNA Sequencing to Identify MicroRNAs in Acute Kidney Injury and Fibrosis

C. V. Gerlach[1,5], K. L. Pellegrini[5], F. L. Craciun[5], K. Ramachandran[5], V. Bijol[5], H. T. Kissick[5] and V. S. Vaidya[1,5]. [1]*Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA;* [2]*Department of Medicine, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA;* [3]*Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA;* [4]*Department of Surgery, Urology Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA and* [5]*Laboratory of Systems Pharmacology, Harvard Program in Therapeutic Science, Harvard Medical School, Boston, MA.*

Establishing a microRNA (miRNA) expression profile in affected tissues provides an important foundation for the discovery of miRNAs involved in the development or progression of pathologic conditions. We conducted small RNA sequencing to generate a temporal profile of miRNA expression in the kidneys using a mouse model of folic acid-induced (250 mg/kg i.p.) kidney injury and fibrosis. From the 103 miRNAs that were differentially expressed over the time course (>2-fold, $p<0.05$), we chose to further investigate miR-18a-5p, which is expressed during the acute stage of the injury; miR-132-3p, which is upregulated during transition between acute and fibrotic injury; and miR-146b-5p, which is highly expressed at the peak of fibrosis. Using qRT-PCR, we confirmed the increased expression of these candidate miRNAs in the folic acid model as well as in other established mouse models of acute injury (ischemia/reperfusion injury) and fibrosis (unilateral ureteral obstruction). *In situ* hybridization confirmed high expression of miR-18a-5p, miR-132-3p and miR-146b-5p throughout the kidney cortex in mice and humans with severe kidney injury or fibrosis. When primary human proximal tubular epithelial cells were treated with model nephrotoxicants such as cadmium chloride (CdCl2), arsenic trioxide, aristolochic acid (AA), potassium dichromate and cisplatin, miRNA-132-3p was upregulated 3.5-fold after AA treatment and 1.5-fold after CdCl2 and cisplatin treatment. These results demonstrate the application of temporal small RNA sequencing and suggest that miR-18a, miR-132 and miR-146b may be important miRNAs during distinct phases of kidney injury and fibrosis progression.

3139 Effect of Cytochrome P450 Isozyme Inhibitors on 1,2,3-Trichloro-5-Nitrobenzene-Induced Nephrotoxicity *In Vitro*

J. Tate[2], C. Tyree[1], D. Pope[1], D. Anestis[1] and G. Rankin[1]. [1]*Marshall University, Huntington, WV and* [2]*West Virginia Wesleyan College, Buckhannon, WV.*

Chloronitrobenzenes are important chemical intermediates in the manufacture of a wide range of dyes, agricultural agents and industrial compounds. Among the four trichloronitrobenzenes evaluated to date, 1,2,3-trichloro-5-nitrobenzene (1,2,3-TC-5-NB) is the most potent nephrotoxicant *in vitro*. Previous studies have suggested that 1,2,3-TC-5-NB may be bioactivated to one or more toxic metabolites by the kidney. The purpose of this study was to begin to explore the role of renal cytochrome P450s (CYPs) in the bioactivation of 1,2,3-TC-5-NB to nephrotoxic metabolites. Isolated renal cortical cells (IRCC) were obtained from male Fischer 344 rats and incubated (~4 million cells/ml; 3 ml) for 30 min with vehicle (dimethyl sulfoxide), a CYP inhibitor, 1,2,3-TC-5-NB (1.0 mM), or the CYP inhibitor + 1,2,3-TC-5-NB. Cytotoxicity was determined by measuring lactate dehydrogenase (LDH) release from cells into media. 1,2,3-TC-5-NB-induced cytotoxicity was reduced by pretreatment with nonspecific CYP inhibitors [piperonyl butoxide (0.1 mM) or metyrapone (1.0 mM)], indicating a role for CYPs in the bioactivation of 1,2,3-TC-5-NB. Pretreatment with more isozyme specific CYP inhibitors, thiotepa (CYP2B1/2 inhibitor, 0.1 mM), diethyldithiocarbamate (CPY2C>2E inhibitor, 0.1 mM), isoniazid (CYP2E1 inhibitor, 1.0 mM), sulfaphenazole (CYP2C family inhibitor, 0.1 mM) or oleandomycin (CYP3A1/2 inhibitor, 0.5 mM), attenuated 1,2,3-TC-5-NB cytotoxicity to varying degrees. These results indicate that CYPs are important in bioactivating 1,2,3-TC-5-NB to one or more toxic metabolites. In addition, multiple CYPs appear to play a role in this bioactivation process. (Supported by NIH grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)

3140 Identifying Predictive Gene Signature and Signaling Networks Associated with Acute Kidney Injury Using Gene Co-expression Modules

M. D. M. AbdulHameed[1], D. L. Ippolito[2], J. D. Stallings[2] and A. Wallqvist[1]. [1]*Department of Defense Biotechnology High Performance Computing Software Applications Institute, Frederick, MD and* [2]*US Army Center for Environmental Health Research, Fort Detrick, MD.*

Understanding the molecular mechanisms and signaling networks of acute kidney injury (AKI) will aid in biomarker development. In this study, we carried out co-expression-based analyses of DrugMatrix, a toxicogenomics database with kidney gene expression data from rats after exposure to diverse chemicals. We used the iterative signature algorithm and exhaustively generated modules using 50 different parameter combinations. We clustered the modules using gene and condition overlap scores and obtained 16 module clusters. Two of the module clusters showed activation in chemical exposures causing kidney injury and mapped well-known AKI marker genes such as Havcr1, Tff3, and Clu. We used the two AKI-relevant module cluster genes along with a random forest approach and developed a gene signature consisting of 30 genes, thus allowing for earlier prediction of kidney injury. The gene signature performed better than well-known biomarkers and had a receiver operating characteristic - area under the curve of 0.91, sensitivity of 79%, and specificity of 90%. Pathway and network analyses of the AKI-relevant modules showed that there is limited information about signaling networks associated with approved pre-clinical AKI biomarker Havcr1. To address this gap, we analyzed the frequently co-expressed genes with Havcr1 in AKI-relevant modules and identified 33 such genes. These genes included Cd44, Plk2, and Macrod1. We further tested the validity of the result by repeating the analyses with randomization of the dataset and by excluding 5% of the data. Finally, we validated the frequently co-expressed genes using external data from Gene Expression Omnibus. Our results showed that co-expression based analysis of toxicogenomics data enabled us to identify new biomarker signatures as well as to gain insight into toxicity pathways. Disclaimer: The views expressed are those of the author(s) and do not constitute endorsement by the US Army or the US Department of Defense.

3141 Differential Expression of Cytochrome P450 Enzymes in Placenta, Fetal Liver, and Adult Liver

J. F. Robinson[3], E. G. Hamilton[3], A. R. Zota[2], J.-S. Park[4], E. Parry[1,4], S. J. Fisher[3] and T. J. Woodruff[3]. [1]*Public Health Institute, Oakland, CA;* [2]*Department of Environmental and Occupational Health, George Washington University, Washington, DC;* [3]*Department of Obstetrics, Gynecology & Reproductive Sciences, University of California, San Francisco, San Francisco, CA and* [4]*Department of Toxic Substances Control, California EPA, Berkeley, CA.*

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants and persist in the environment. PBDEs accumulate in human fetal and adult tissues with unknown consequences. Proposed as biomarkers of PBDE exposure in rodent models, cytochrome P450 enzymes (CYPs) are responsible for metabolism of PBDEs to their hydroxylated forms. However, during human pregnancy, the expression of CYPs in fetal tissues is poorly understood. Therefore, in this study, we assessed the expression of nine CYP isoenzymes in placentas (n=5), fetal (n=85) and adult (n=10) livers by using qRT-PCR to determine levels within and across the groups. CYP profiles were highly organ-dependent. Seven of the nine CYPs were expressed at significantly lower levels in fetal livers or placentas vs. adult livers. For example, CYP2E1 levels were ~10,000X lower in the fetal organs examined as compared to adult livers ($p<0.01$). Uniquely, CYP19A1 was expressed at significantly higher levels in placentas vs. fetal or adult livers. Comparisons within the fetal liver group suggested significant correlation of CYP1A1 with gestational age with the most variable CYPs being 2C19 and 2E1. No sex differences were observed between male and female liver samples. Variations in expression profiles between fetal and adult tissues may underlie differences in metabolism, and potentially, their susceptibility to PBDEs. In ongoing studies, we are investigating the distribution of 17 PBDEs in matched 2nd trimester maternal serum, placental, and fetal liver samples. The goal is to identify and evaluate potential molecular correlates (e.g., CYPs) of PBDE exposure in these compartments.

3142 Cytochrome P450 1B1: A Key Regulator of the Cellular Oxidative State

N. Sheibani[4], C. Marcus[2], C. R. Jefcoate[4] and C. M. Sorenson[4]. [1]*Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI;* [2]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR;* [3]*Ophthalmology and Visual Sciences, University of Wisconsin-Madison, Madison, WI and* [4]*Pediatrics, University of Wisconsin-Madison, Madison, WI.*

CYP1B1 is a member of Cytochrome P450 family of proteins with important roles in developmental processes. Mutations in human CYP1B1 gene have been linked to the pathogenesis of congenital glaucoma. However, the detailed mechanisms remain unclear. We have shown that Cyp1b1 expression is essential during pathological angiogenesis driven by hyperoxia-mediated ischemia in developing retinal vasculature, which can be relieved by antioxidants. We showed that increased thrombospondin-2 expression is responsible for the angioinhibitory activity of oxidative stress. These activities are consistent with constitutive expression of Cyp1b1 in endothelial cells, and more predominantly in perivascular supporting cells, which maintain the cellular oxidative state especially under hyperoxia conditions. Furthermore, we showed that Cyp1b1-deficiency results in degeneration of the trabecular meshwork during development of the ocular drainage system resulting in increased intraocular pressure and glaucoma. This was associated with decrease production of periostin, a molecule with important role in development and maintenance of collagen fibrils that form and support the trabecular meshwork function. Together, our studies demonstrate an important role for Cyb1b1 in regulation of cellular oxidative state, especially under hyperoxic conditions, with adverse effects on various cell functions.

3143 Role of CytochromeP450 (CYP)1A in Hyperoxic Lung Injury: Analysis of the Transcriptome and the Proteome

K. Lingappan, S. Maity, J. Weiwu, L. Wang, X. Couroucli, C. Coarfa and B. Moorthy. *Baylor College of Medicine, Houston, TX.* Sponsor: B. Moorthy.

Background: Hyperoxia contributes to lung injury in experimental animals and lung diseases such as ARDS in humans. Cytochrome P450(CYP)1A enzymes are differentially protective in hyperoxic lung injury. The molecular pathways that modulate the protective effects of CYP1A in hyperoxic lung injury (HLI) remain largely unknown. Objective: To characterize CYP1A1/1A2 genotype specific differences in the tran-

scriptome and proteome in acute hyperoxic lung injury using microarray and reverse phase protein array (RPPA). Design/Methods: Wild type (WT), Cyp1a1-/- and Cyp1a2-/- (8-10 wk, C57BL/6J background) mice were exposed to hyperoxia (FiO2>0.95) for 48 hours. Gene expression in lung tissue was studied using the Illumina Mouse WG-6 v2.0 expression beadchip. Preprocessed gene expression data was normalized using the lumi package, and analyzed using the R statistical system. A fold change ≥ 1.2 and false discovery rate < 20% was used to define differentially expressed genes (DEGs). Analysis of enriched pathways and transcription factors was done using hypergeometric distribution ($p<0.05$) and the compendium curated by the Molecular Signature Database (MSigDB). Results: A total of 304 (up-regulated (UR): 160, down-regulated (DR): 144) and 174 (UR: 59, DR: 115) genes were differentially expressed following hyperoxia when compared to WT mice in Cyp1a1-/- and Cyp1a2-/- mice respectively. Comparison of DEG profiles in hyperoxia exposed animals identified 171 genes unique to Cyp1a1-/- and 119 unique to Cyp1a2-/- mice. Cholesterol biosynthesis and lipid metabolic process (Mvd, Fdps, Pmvk, Dhcr24) was up regulated and the p53 signaling pathway (Cdkn1a, Atm) was down regulated in Cyp1a1-/- mice. In Cyp1a2-/- mice, retinol metabolism (Rdh11, Hsd11b1) was up regulated, while the extracellular matrix receptor interaction pathway (CD44, Hspg2) was down regulated compared to WT mice. The RPPA data showed up regulation of PTEN (1.4 fold) and VEGFR2 (1.3 fold) in Cyp1a1-/- mice and Bcl2 (2.8 fold), cleaved PARP (2.7 fold), and Rad50 (2.6 fold) in Cyp1a2-/- mice. Conclusions: DEGs involved in cholesterol/lipid metabolism and p53 signaling pathway in Cyp1a1-/- mice and retinol metabolism and ECM-receptor interaction in Cyp1a2-/- mice, may explain the differences in susceptibility to HLI. These findings suggest new pathways that need to be investigated as possible therapeutic targets for acute lung injury in humans.

PS 3144 RNA-Seq Reveals True Quantification of Xenobiotic Processing Genes in Various Sections of the Intestine Compared to Liver of Mice

Z. D. Fu, F. P. Selwyn, J. Y. Cui and C. D. Klaassen. *University of Washington, Seattle, WA.*

Previous reports on the tissue distribution of the xenobiotic processing genes (XPGs) have limitations in that (1) many non-P450 phase-I enzymes and transcription factors have not been investigated, and (2) one cannot compare the real abundance of multiple genes by conventional mRNA quantification methods. The purpose of this study was to compare the mRNA abundance of all major XPGs in liver and intestine using RNA-seq. The mRNA profiles of 304 XPGs were systematically examined in various sections of the intestine, namely the duodenum, jejunum, ileum, and large intestine, in addition to liver of adult male C57BL/6 mice. Over 80% of these XPGs had differential expression among tissues, which partitioned into liver-, small intestine-, and large intestine-predominant expression patterns as revealed by two-way hierarchical clustering analysis. Among these XPGs, 54% were highest expressed in liver, 21% in duodenum, 4% in jejunum, 6% in ileum, and 15% in large intestine. Among all the XPGs, the highest expressed gene in liver was Mgst1, in duodenum Cyp3a11, in jejunum and ileum Ces2e, and in large intestine Cyp2c55. Genes in the same gene family usually exhibited highly distinctive tissue-specific expression patterns. It is noteworthy that the percentages of individual gene mRNAs in cumulative mRNAs of all genes in the same family/category were usually similar in the three sections of the small intestine. In conclusion, the present study is among the first to provide true quantification of mRNA abundance of all major XPGs in various sections of mouse intestine and liver. Such knowledge will provide mechanistic explanation for tissue-specific biotransformation and tissue-specific toxicity of drugs and other xenobiotics.

PS 3145 Prolonged Culturing of Human Hepatocytes in Human Plasma for P450 Induction and *In Vitro* Hepatotoxicity Studies

Q. Yang, U. Doshi, K. Amaral, C. Loretz and A. P. Li. *In Vitro ADMET Laboratories LLC, Columbia, MD.*

We report here a novel finding that human hepatocytes can be cultured for an extensive period in 100% human plasma. Cryopreserved human hepatocytes from 12 donors were plated in collagen -coated plates in normal incubation media. After 4 h of plating, Matrigel was added to create a Matrigel-collagen sandwich culture. On the day after culturing, medium was changed to 100% human plasma (pooled from plasma from 5 male and 5 female donors). The morphology of the human hepatocytes changed drastically on the first 3 days of culture, with rounded cell shape and extensive intracellular vesicles. However, from day 4 onwards, the hepatocytes began to assume a normal, epithelial cell morphology with diminished intracellular inclusions. After day 7, the cells had a stabilized epithelial morphology with clear cytoplasm which remained unchanged up to the longest incubation evaluated of 30 days. Cellular ATP contents remained constant from day 7 to day 30. The human plasma cultured human hepatocytes (HPHH) were applied towards prolonged *in vitro* hepatotoxicity studies. Aflatoxin B1 (AFB1), a hepatotoxicants known to require metabolic activation to cytotoxic and genotoxic metabolites, was found to be cytotoxic to human hepatocytes cultured in human plasma. Using cellular ATP contents as viability endpoint, AFB1 had an IC50 value of 19.3 uM after 2 days of treatment. The IC50 value decreased to 1.1 uM after 10 days of treatment. To further demonstrate that HPHH retained key liver properties, human hepatocytes from 4 donors were cultured in human plasma for 5 days, followed by a 2 day treatment with model P450 inducers. Induction of CYP1A2, CYP2B6 and CYP3A4 was observed for the model inducers of the isoforms, namely, omeprazole, phenobarbital, and rifampin, respectively. Our studies suggest that HPHH may represent an *in vitro* hepatocyte system which may allow direct extrapolation of *in vitro* findings to *in vivo* effects based on *in vivo* plasma drug concentrations.

PS 3146 Human Enterocytes: Isolation, Cryopreservation, Characterization, and Application in the Evaluation of Drug-Food Interactions

A. P. Li, C. Loretz, Q. Yang and U. Doshi. *In Vitro ADMET Laboratories LLC, Columbia, MD.*

As of now, there are no appropriate cell-based experimental systems for the evaluation of intestinal metabolism. The existing cellular models consist of primary cultured enterocytes from intestinal fragments, which, while retaining some barrier properties, have greatly diminished drug metabolizing activities, especially that of CYP3A4 which is known to be expressed in the small intestines. To overcome this deficiency, we embarked on the isolation and cryopreservation of enterocytes from human small intestines. Human small intestinal segments (duodenum and proximal jejunum) were obtained from organ donors. The lumen of the small intestine segments was washed extensively to remove extracellular contents followed by collagenase digestion to release the enterocytes. The isolated enterocytes were purified by differential centrifugation and cryopreserved in liquid nitrogen. The enterocytes were cryopreserved directly after isolation, without culturing, to allow the retention of drug metabolizing activities. Post-thaw viability of the cryopreserved enterocytes was consistently over 80% based on trypan blue expression. RT-PCR quantification demonstrated the expression of P450 genes: CYP3A4 (59.4% of total), CYP2C9 (22.5%), CYP2C19 (12.5%), CYP3A5 (5%), detectable but less than 1% of CYP2B6, CYP2C8, CYP2D6, and CYP1A2, and no detectable expression of CYP2E1. CYP3A4 activity of the enterocytes was found to similar to that of human hepatocytes. A proof-of-concept study for the application of human enterocytes as an *in vitro* experimental system for the evaluation of food-drug interaction was performed with grapefruit juice (GFJ). Direct incubation of the enterocytes with GFJ led to dose- and time-dependent inhibition of CYP3A4 activity. Our results showed that cryopreserved human enterocytes represent a relevant *in vitro* experimental system for the evaluation of intestinal drug metabolism and food-drug interactions.

PS 3147 Regional Distribution of CYP2A13 and CYP2F1 mRNAs in Human Lung and in the Lungs of CYP2A13/2F1-Humanized Mice

L. S. Van Winkle[3], P. Edwards[3], L. Li[2], H. Wu[2], N. Kovalchuk[2], Q.-Y. Zhang[2] and X. Ding[1]. [1]*SUNY Polytechnic Institute, Albany, NY;* [2]*Wadsworth Center, New York State Dept of Health, Albany, NY and* [3]*Center for Health and the Environment, UC Davis, Davis, CA;* [4]*Vet Med: Anatomy, Physiology and Cell Biology, UC Davis, Davis, CA.*

P450-mediated metabolism is a prerequisite for naphthalene cytotoxicity in the lung. The major mouse CYP that metabolizes naphthalene, CYP2F2, is localized to epithelial Club cells that are found throughout the conducting airway of the mouse. Areas of greatest cytotoxicity in the mouse airway, terminal bronchioles, have the greatest expression and activity of CYP2F2. In the human lung, the CYP2F2 ortholog CYP2F1, as well as CYP2A13, are expressed; both metabolize naphthalene. However, the classic Club cells have a more restricted distribution in the human lung than in rodent lungs, confined to distal airways. Knowledge of species differences in the distribution of enzymes involved in naphthalene bioactivation is thus important in understanding risks from naphthalene exposure. The goal of this study was to define the distribution of CYP2A13 and CYP2F1 mRNAs in CYP2A13/2F1-humanized transgenic mouse lung and compare this result to 1) normal expression of CYP2F2 in the mouse and 2) normal expression of the CYP2A13 and CYP2F1 genes in the human lung. Lung tissue was laser-capture microdissected, and mRNA for CYP2F2, CYP2A13 and CYP2F1 were measured. Three groups of mice were examined: Null mice (Cyp2abfgs-null) that

lack normal expression of mouse CYP2F2, wild type mice (WT) that have the normal mouse CYP expression, and humanized mice (CYP2A13/2F1-transgenic and Cyp2abfgs-null) that contain both of the human genes on the Null mouse background. Three regions were examined: terminal bronchioles, proximal bronchioles and parenchyma; whereas airways and alveoli were compared in a limited set of human lung biopsy samples. Our results to date demonstrate that CYP2A13 and CYP2F1 mRNAs are preferentially expressed in the airways, both in human lung and in the lungs of the CYP2A13/2F1-humanized mice. In comparison to CYP2F2, which demonstrates enrichment in terminal bronchioles of the distal airways, CYP2A13 and CYP2F1 were expressed throughout both proximal and distal airways in the humanized mice. Further studies are underway to determine whether CYP2A13 and CYP2F1 are also expressed throughout both proximal and distal airways in human lung or are restricted to more distal airways. Supported by NIH grant ES020867

PS 3148 Stabilized and Enhanced CYP450 Enzyme Activity in Cultured Human Primary Hepatocytes is Conferred by ReproHP Medium

P. E. Cizdziel[3], M. Inamura[3], R. Vardaro[5], B. Annand[5], R. Akahira[3], Z. Y.-C. Lin[3], K. Tamura[3], T. Watanabe[3] and T. Kiyono[1]. *[1]National Cancer Research Institute, Tokyo, Japan; [2]Research & Development, ReproCELL Inc., Kohoku-ku, Yokohama, Japan; [3]Technical Marketing, ReproCELL Inc., Kohoku-ku, Yokohama, Japan; [4]Technical Marketing, Stemgent Inc., Boston, MA and [5]Technical Product Manager, Stemgent Inc., Boston, MA.*

The use of cultured human primary hepatocytes to evaluate drug metabolism *in vitro* is widely adopted with guidelines issued by many global regulatory authorities including the FDA and EMA. However, in culture, primary hepatocytes rapidly lose their ability to express key metabolic enzymes, such as the cytochrome P450 (CYP) gene family. Typically, CYP activity declines to undetectable levels in about 96 hours. This instability causes logistical problems and higher costs for drug screening assays. In this study, we developed a new cell culture medium that maintains high CYP activities of primary hepatocytes even at 96 hours in culture. Using rational design we first screened many combinations of different physical parameters, chemical inhibitors and biological compounds (nutrients, growth factors, etc.) for the ability to increase CYP3A4 activity (qPCR assay) in culture. Ten different promising medium compositions were further characterized by the P450-Glo™ CYP3A4 assay kit with Luciferin-IPA after 96 hours of cell cultivation. One novel composition was selected from the candidates based on performance, stability, cost and other criteria. Activity of CYP3A4 and other CYP enzymes were measured by using the qPCR assay. Hepatocytes cultured with our new medium showed higher levels of activity for all CYP enzymes tested compared to the most popular brand of medium. For further improvement, we seeded human primary hepatocytes on top of the Alvatex scaffold (Reinnervate, Inc.); providing a 3D cultivation system to test with the new medium. As judged by qPCR, the basal level and induced activity of CYP3A4 in 3D culture was elevated relative to monolayer culture. Furthermore, CYP activity of ReproHepato (iPS cell-derived hepatocytes), could also be improved. In conclusion, the new formulation, branded ReproHP Medium, showed high performance for *in vitro* drug metabolism studies with improved CYP activity, induction levels, and stability of expression in both 2D and 3D culture formats, while maintaining a highly distinct cobblestone morphology characteristic of mature human hepatocytes.

PS 3149 Separate Assessment of Basal and Induced Cyp1B1 Activity in the Presence of Cyp1A1. Relationship to Cyp1B1 Involvement in Physiological and Toxicity Processes

A. A. Almeldin[2,3], C. M. Rondelli[1,3], M. Maguire[3], M. C. Larsen[3] and C. R. Jefcoate[1,3]. *[1]METC, University of Wisconsin-Madison, Madison, WI; [2]Physiology Department, Tanta Faculty of Medicine, Tanta, Egypt and [3]CRB, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI.*

Benzo[a]pyrene (BP) but not 7,12 dimethylbenz[a]anthracene (DMBA) extensively stimulates many of these cytokines in BM within 6h. This process is attenuated in Cyp1A1-ko mice. Intermediate increases in these cytokines are produced by depletion of the Cyps in absence of PAH (Cyp1B1-ko > Cyp1A1-ko). These changes indicate that endogenous substrates of Cyp1B1 and Cyp1A1 play a significant role in the basal generation of cytokines such as Tnfα, IL6, CXCL10 and Ifn-γ in BM. These CYP activities are extensively induced and inhibited by pollutants (PAHs, PCBs etc) and dietary components (flavonoids), suggesting alternative means for affecting cytokine levels and inflammatory processes. To evaluate this CYP-mediated metabolism more fully in live cells, we optimized ethoxy- and benzyloxy-resorufin (EROD, BROD) fluorimetric assays for selectivity to respectively CYP1A1 and CYP1B1. V79 fibroblasts expressing similar levels of either recombinant human CYP1B1 or CYP1A1 were compared. EROD is 50 times more active for CYP1A1 whereas BROD has equal activity with each form. EROD activities were linear for 6h while BROD activity was maintained for only 3h. This process was evaluated for suicide inhibition by the BROD product (benzaldehyde)and for cytotoxicity. Specific CYP1B1 assessment was optimized by additional use of tetramethoxystilbene (TMS) in combination with BROD. CYP1B1 activity was determined as the proportion of BROD activity that is sensitive to TMS inhibition (1uM). CYP1A1 activity was determined as EROD activity that is insensitive to TMS. To test this method, basal and TCDD induced activities for CYP1B1 and CYP1A1 were distinguished in human breast cell lines, each in comparison to transcript levels determined by qPCR. Selective inhibition by certain flavonoids (Chrysin, Apigenin) were tested. Mouse Cyp1B1 has been shown to control systemic regulation of obesity and liver gene expression. Many of the conserved gene changes represent a suppression of inflammatory processes. This BROD/EROD assay procedure was being applied to assessment of Cyp1B1 activity in mouse BM mesenchymal progenitors, monocytes, and macrophage in response to cytokine and PAH stimulation.

PS 3150 Evaluation of Xenobiotic Metabolism Inhibition with 23 Polycyclic Aromatic Compounds in Primary Human Hepatocyte Suspensions

W. Qu, M. J. DeVito, S. S. Fersuson and C. V. Rider. *Division of the National Toxicology Program, Research Triangle Park, NC.*

Suspensions of primary human hepatocytes (PHHs) are widely recognized as the gold standard *in vitro* model system to study drug metabolism. PHHs retain levels/proportions of cytochromes P450 (CYPs) known to be responsible for the metabolism of xenobiotic compounds. CYP1A2, CYP2B6, CYP3A4 and CYP2C9 collectively are involved in the metabolism of > 60% of drugs. Therefore, inhibition of these enzymes can play a prominent role in xenobiotic metabolism clearance and contribute to metabolically-activated toxicity (i.e. protein adducts, mechanism-based inhibition). We evaluated 23 polyaromatic compounds (PACs) in suspensions of PHHs using luminescent P450-Glo assays. Cryopreserved human hepatocytes pooled from 50 donors were thawed and incubated at 50,000 cells per well with viabilities of ~85% (cellometer measurement with AOPI staining). 23 PACs were initially evaluated at 50 µM for CYP1A2, CYP2B6, CYP3A4 and CYP2C9 using isoform-specific P450-Glo substrates at 1 and 4 hours. CYP1A2 was inhibited by the most of 23 PACs at both time points. Specifically, dibenz[a,h]anthracene, benzo(a)pyrene, phenanthrene, acenaphthenequinone, fluorene, benz(a) anthracene significantly inhibited CYP1A2 at 1.9, 6.6, 3.4, 8.7, 5.2 and 211-fold, respectively. Concentration-response analysis revealed fluvoxamine, a potent positive control inhibitor of the human CYP1A2, inhibited CYP1A2 at 5.9-fold with mean IC50 values of 0.042 µM. Omeprazole (100 µM), a potent inducer of the human CYP1A2 as a control, produced a 2.8-fold increase in CYP1A2 with mean EC50 of 14.11 µM. Benzo(a) pyrene (IC50, 1.66 µM), phenanthrene (IC50, 5.88 µM), and benz(a)anthracene (IC50, 1.54 µM) markedly inhibited CYP1A2 while dibenz[a,h] anthracene, acenaphthenequinone and fluorine moderately inhibited CYP1A2. These data were evaluated against QSAR models for CYP1A2 inhibition (ADMET-Predictor) and in context with liver enzyme induction evaluations to further interpret the actions of PACs on human liver xenobiotic metabolism pathways.

PS 3151 Roles of Cyp1B1 and XPC in the Suppression of Hematopoietic Progenitor Cells by PAHs

C. M. Rondelli[3], M. C. Larsen[3], A. N'jai[3], C. Mao[3], C. J. Czprynski[3] and C. R. Jefcoate[3]. *[1]Cell & Regenerative Biology, University of Wisconsin-Madison, Madison, WI; [2]Cell & Regenerative Biology, Molecular & Environmental Toxicology Center, University of Wisconsin-Madison, Madison, WI and [3]Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI.*

Hematopoietic progenitor cells in bone marrow (BM) are selectively suppressed in response to polyaromatic hydrocarbons (PAH), such as DMBA. This directly links to suppression in mature (BM) populations 48h later, and also to thymic and splenic cell losses. This depletion is dependent on Cytochrome P450 1B1 (Cyp1B1), but not the arylhydrocarbon receptor (AhR). The BMS2 cell line, in which Cyp1B1 is also AhR resistant, models Cyp1B1 functions in BM mesenchymal progenitor cells. Cyp1B1 is an essential participant in the p53 pathway, which we have shown responds differentially to PAHs in BMS2 cells (DMBA > benzopyrene). To study the mechanism behind this effect, we set up an *in vitro* cell culture system that mimics parts of the bone marrow microenvironment. This comprised of a mesenchymal multipotential progenitor cell (MMPC) support component and freshly isolated primary BM cells (BMCs). Our

hypothesis is that hematopoietic progenitor cell expansion capacity is primarily inhibited by PAH metabolites released from MMPCs that damage DNA in the progenitor cells. Progenitor cell expansion activity was measured by colony formation unit [CFU] assays for erythrocyte, pre-B lymphocyte, and granulocyte-monocyte cellular lineages. These responses are suppressed by PAHs *in vivo* within 6h. MMPC released factors that maintained hematopoeitic progenitor CFU activity in the absence of cells. Direct DMBA metabolism and toxicity within the progenitor cells were assessed using primary BMCs derived from mice lacking either Cyp1B1 or a DNA damage response gene (nucleotide excision repair pathway gene XPC). DMBA toxicity after 24h was assessed in BMCs cultured either with MMPCs or in MMPC conditioned medium, using the CFU assays. XPC deletion in BMCs greatly enhanced DMBA potency (EC50 0.05µM vs 1µM). DMBA also inhibited BMC progenitors cultured in MMPC conditioned medium, though at a much reduced potency (EC50 3µM). This inhibition was not seen with Cyp1B1 deletion BMCs. These findings support a model in which MMPCs are both the source of hematopoietic support factors, and Cyp1B1-mediated metabolites that target progenitor cell DNA, resulting in DNA damage that is repaired by XPC.

3152 Attenuation of Hyperoxic Lung Injury in *Cyp1b1-/-* Mice

A. C. Veith[1,2], B. Bou Aram[1], W. Jiang[1], L. Wang[1], C. Chu[1], X. I. Couroucli[1] and B. Moorthy[1,2]. [1]*Department of Pediatrics-Newborn, Baylor College of Medicine & Texas Children's Hospital, Houston, TX and* [2]*Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX.*

Rationale: Supplemental oxygen (hyperoxia) is a life-saving intervention provided to patients suffering from respiratory distress, such as adults with acute respiratory distress syndrome (ARDS) and bronchopulmonary dysplasia (BPD) in premature newborns. Phase I and II metabolism enzymes and have previously been implicated in altering susceptibility to hyperoxia induced lung injury. Hypothesis: The purpose of this study was to test the hypothesis that cytochrome P450 1B1 (CYP1B1) is an important mediator promoting hyperoxic lung injury in an adult mouse model. Methods: Ten week old C57BL6 (WT) and Cyp1b1-/- male mice were exposed to hyperoxia (>95% oxygen) for 24-72 hours or in room air. Lung injury was assessed by relative lung weight increases and histology. RT-PCR and Western Blot were used to quantitate expression of CYP1A1/1A2 and CYP1B1. Results: Cyp1b1-/- mice had significantly attenuated lung weight increases due to hyperoxia exposure (P=0.01), specifically at both 48 (P=0.04) and 72 hours (P=0.017) of hyperoxia, indicating reduced lung injury. Histological sections confirmed reduced pulmonary edema in Cyp1b1-/- mice. Immunohistochemical stained lung sections suggest altered neutrophil recruitment kinetics in Cyp1b1-/- mice compared to WT. Furthermore, RT-PCR indicates that Cyp1b1-/- mice have increased pulmonary Cyp1a1 (P=2.9x10-05) throughout the hyperoxia exposure, and prolonged hepatic Cyp1a2 gene expression at 48 hours (P=0.016), both of which have been shown to be protective in mouse models of hyperoxic lung injury. Interestingly, this study has also identified that Cyp1b1 is induced in the lungs during hyperoxia (P=0.003) by both RT-PCR and Western blot. Conclusions: This study provides the framework to continue further research into the mechanism by which CYP1B1 alters susceptibility to hyperoxic lung injury and to develop CYP1B1-directed treatment strategies or therapeutics to prevent and/or treat ARDS/BPD.

3153 Comparison of CYP2B6, 2C8, 2C9, 2C19, 3A4 and 3A5 Induction in 3 Donors of Plated Human Hepatocytes by 15 Compounds

D. M. Stresser[1], R. Patel[1], R. J. Clark[1], T. Ho[1], S. K. Trisdale[1], Y. Fang[2] and J. G. Zhang[1]. [1]*Corning Life Sciences, Woburn, MA and* [2]*Research and Biochemical Technologies, Corning Incorporated, Corning, NY*. Sponsor: C. Crespi.

We previously reported that calibration curve-based approaches generated from CYP3A4 mRNA induction data in human hepatocytes can be used to predict CYP3A4 induction in humans. Here, same set of samples were analyzed to evaluate induction of CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A5 mRNA. Hepatocytes from three lots were treated with a set of 15 CYP3A4 *in vitro* inducers for 2 days, typically over 8 concentration points to enable calculation of EC50 and Emax. After treatment, total RNA was isolated and CYP mRNA expression was determined by real-time RT-PCR analysis. Based on Emax values, the overall rank order of induction response was 2B6>3A4>2C8>3A5>2C9>2C19, which held for all 3 donors. Rank order and absolute differences often varied by compounds. For example, rifampicin (RIF) induced CYP3A4 by a mean of 10-fold, but was essentially unable to induce CYP3A5. In contrast, phenobarbital (PB) induced CYP3A4 by 7.2-fold and CYP3A5 by 4.8-fold. Nifedipine induced CYP3A4 by 5.6-fold, and induced CYP2C9 by 3.9-fold, whereas RIF and PB induced CYP2C9 by < 2.5-fold. Interestingly, EC50 values with CYP3A5 mRNA induction were generally much higher than CYP3A4 (5.5 ± 6.1 fold for 14 compounds that induced CYP3A5). In contrast, EC50 values forCYP2B6, CYP2C8 and CYP2C9 were modestly higher (1.7 ± 1.3 to 2.9 ± 4.4-fold greater). Our results demonstrate that induction profiles of CYP2B, 2C and 3A enzymes, that are mediated largely or in part by PXR, can vary substantially by compound and that the response is not simply a scaled value of the CYP3A4 induction response. Finally, these results underscore that induction response of certain enzymes not typically considered (e.g. CYP2C8 and CYP3A5) in drug development can be significant.

3154 Oral Prenatal Administration of the Cytochrome P450 (CYP)1A/1B Inducer, Beta-Napthoflavone (BNF), Attenuates Hyperoxic Lung Injury in Newborn Mice: Implications for Bronchopulmonary Dysplasia (BPD)

X. I. Couroucli, Y. W. Liang, K. Lingappan, A. Veith and B. Moorthy. *Pediatrics, Baylor College of Medicine, Houston, TX.*

Background: Supplemental oxygen is frequently administered to newborns with pulmonary insufficiency. However, prolonged hyperoxia contributes to the development of bronchopulmonary dysplasia (BPD) in preterm human infants. Hypothesis: Prenatal treatment of newborn mice with beta-napthoflavone (BNF), followed by exposure to hyperoxia, would alleviate lung injury by mechanisms involving CYP1A or 1B1 enzymes. Methods: Timed pregnant mice (C57BL/6J) were treated orally with the vehicle corn oil (CO) or BNF (80 mgkg) on gestational days (18-20), and pups born to these mothers were maintained in room air or exposed to hyperoxia (> 95% O2) for 48, 96 or 120 h. The mice were sacrificed on day 5 of life. Expression of the CYP1A1/2 and 1B1 was measured by RT-PCR and Western blots. Lung injury was assessed by determining lung weight/body weight (LW/BW) ratios and histopathology. Results: Mice born to CO-treated mothers showed significant lung injury and alveolar simplification at all three time points of hyperoxia. However, newborn mice born to BNF-treated mothers showed attenuation of lung injury and increased alveolarization, as determined by measuring lung weight/body weight (LW/BW) ratios and histology respectively. The most striking observation was that both pulmonary and hepatic CYP1B1 (not CYP1A1/2) were markedly induced in newborns born to BNF exposed mothers after 48 h of hyperoxia, and that correlated with less lung injury. After 5 days of hyperoxia, the pattern of expression was reversed. Conclusions: Our observations support the hypothesis that prenatal oral BNF protects newborn mice against hyperoxic lung injury by induction of the CYP1B1 and CYP1A1/2 in a temporal fashion. Future studies on the role of CYP1A/1B1 enzymes in oxygen-mediated lung injury could lead to the development of rational strategies in the prevention/treatment of BPD in premature infants.

3155 The Impact of Hepatic Mitochondrial Cyp2e1 Localization in Butadiene-Exposed Mice

J. Hartman[5], G. P. Miller[5], A. A. Caro[1], W. C. Spear[3], S. Miller[3], L. M. Hallberg[3], B. T. Ameredes[2] and G. Boysen[5]. [1]*Hendrix College, Little Rock, AR;* [2]*University of Texas for Medical Branch, Galveston, TX;* [3]*University of Texas Medical Branch, Galveston, TX;* [4]*Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR and* [5]*Environmental and Occupational Health, University of Arkansas for Medical Sciences, Little Rock, AR.*

Cytochrome P450 2E1 (Cyp2e1) metabolically activates many small molecule pollutants, drugs, and other toxicants into reactive metabolites, yet the relevance of those processes in toxicity remains unclear. Although Cyp2e1 is typically studied in the endoplasmic reticulum, it also localizes to mitochondria where its activity leads to mitochondrial protein adducts and dysfunction during acetaminophen metabolism. Similarly, we hypothesize that CYP2E1 levels and activity correlate with mitochondrial DNA damage/protein damage and subsequent mitochondrial dysfunction following exposure to butadiene (BD). We are testing this hypothesis using a genetically diverse population of 60 mouse strains exposed to air or 2, 20, and 200 ppm BD. Mitochondrial Cyp2e1 activity in the control group revealed a >5-fold variability in basal 4-nitrophenol oxidation activity (0.29 to 1.5 nmol/min/mg protein). For the 200 ppm BD exposed mice, initial studies demonstrated a 35% increase in CYP2E1 activity (p=0.072 student's paired t-test) with increased variance in the BD-exposed mice (p<0.01 student's paired t-test). This variance reflects non-uniform effects across the mouse strains; Cyp2e1 activities in BD-exposed mice ranged from 36% to 450% of controls. These findings suggest that genetic variations among the mice impact Cyp2e1 activity following exposure BD. Studies on the mitochondrial consequences of Cyp2e1 activity in 12 strains showed that BD exposure significantly in-

creased (70% increase, p=0.019) mtDNA copy number with no change in mitochondrial integrity. The results implicate an increase in mitogenesis and/or mtDNA synthesis in BD-exposed mice. Ongoing experiments are expanding these studies to all 60 strains of mice, including analysis of mRNA and protein levels of mitogenesis-related genes, and determining the concomitant effects of BD exposure on mitochondrial respiratory complex activities. Together, the results of this study provide the first report of the consequences of mitochondrial Cyp2e1 localization after BD exposure.

3156 Gender Differences in Response to a High Fat Diet in Cyp3a-Null Mice

R. Kumar, E. J. Litoff, W. S. Boswell and W. S. Baldwin. *Biological sciences, Clemson University, Clemson, SC.*

Recent studies have indicated a role for the pregnane X-receptor and xenobiotic detoxifying CYPs in fatty liver disease or obesity. Therefore, we examined whether the lack of Cyp3a, the most predominant hepatic CYP, increases obesity and fatty liver disease. Cyp3a-null mice fed a normal diet show significant increases in Cpt1a regardless of gender (18-123X) and female mice also show an increase in FATP. Following 8-weeks of exposure to a 60% high-fat diet (HFD), males repressed Cpt1a 50%, but not females. Males fed a HFD also showed increased expression of FATP, and repressed SREBP1 and FASN. Surprisingly, Cyp3a-null females provided a HFD gained 50% less weight than WT females. However, Cyp3a-null males initially gained more weight than WT males but they did not continue this trend over eight weeks. In parallel to the weight data, Cyp3a-null females recover faster than WT females from a glucose challenge. Males show no difference in glucose tolerance between the groups. Insulin tolerance tests did not show differences between the treatment groups, indicating that the glucose tolerance is not due to perturbations in the ability of mice to respond to insulin. Serum concentrations of adiponectin, the anti-obesity hormone, are 60% higher in Cyp3a-null females than WT females in congruence with the lower weights and faster glucose response in female Cyp3a-null mice. In conclusion, the lack of Cyp3a has a positive effect on acclimation to a HFD in females with few significant physiological consequences in males although there appears to be compensatory biochemical responses. Keywords: Cyp3a-null mice; High fat diet; Gender differences, obesity

3157 Using Budding Yeast to Assess P450-Dependent Toxicity of Breast Cancer-Associated Chemicals

R. S. Zeidan[2], R. Ma[4], R. Rudel[1], J. Ackerman[1], A. Loguinov[2], M. Tagmount[2], C. Vulpe[2,4] and M. Fasulo[3]. [1]*Silent Spring Institute, Newton, MA;* [2]*Center of Environmental and Human Toxicology, University of Florida, Gainesville, FL;* [3]*College of Nanoscale Science and Engineering, State University of New York Polytechnic Institute, Albany, NY and* [4]*Nuitritional Sciences and Toxicology, University of California - Berkeley, Berkeley, CA.*

The incidence of breast cancer is correlated to both genetic factors and exposure to environmental and hormonal agents. Many of these agents are not genotoxic per se but are metabolically activated by P450 enzymes that generate highly reactive genotoxic epoxides. Nonetheless, P450 enzymes may also be protective, facilitating excretion of toxic compounds by rendering them hydrophilic. To discern which P450 enzymes activate carcinogens or may be protective against breast cancer-associated chemicals, we have used budding yeast to express individual P450 genes. We have individually expressed CYP1A1, CYP1A2, CYP1B1, and CYP3A4 in a pdr5 strain, defective in export of xenobiotics, and in a rad4 rad51 mutant, defective in both nucleotide excision and recombinational repair. We have studied the sensitivity of these strains to 117 chemicals, including estrone, estradiol, testosterone, zearalenone, and dimethylbenz(a)athracene. We utilized a 96-well plate format to monitor growth for high throughput studies. While dimethylbenz(a) anthracene is toxic regardless of the CYP expressed, sensitivity to testosterone is CYP-dependent, and resistance to zearalenone is enhanced by expression of CYP1A1. These studies indicate that human CYPs can enhance either genotoxicity or resistance to xenobiotics and illustrate the utility of the yeast system as a tool for high throughput studies of P450-activated agents.

3158 Role of CYP1A2 in the Regulation of Hepatic and Pulmonary Cytochrome P450 (CYP)1A1 Expression in 3-Methylcholanthrene Treated Mice

P. Maturu, W. Jiang, L. Wang, C. Chu and B. Moorthy. *Baylor College of Medicine, Houston, TX.*

Background: 3-Methylcholanthrene (MC) is one of most potent polycyclic aromatic hydrocarbons (PAHs) present in cigarette smoke, diesel exhausts, and charcoal broiled meats, etc. Cytochrome P450 (CYP) 1A enzymes play key roles in the activation of PAHs to carcinogenic metabolites. Hypothesis: In this study, we tested the hypothesis that CYP1A2 plays a mechanistic role in the induction of pulmonary and hepatic CYP1A1 by MC. Methods: Wild type (WT) (C57B6) and Cyp1a2_null mice were divided into two groups. Group I was treated with vehicle corn oil (CO) (8 ml/kg) and group II was treated with four doses of MC (100 µmol/kg), i.p. once daily for 4 days. Four animals from each group were sacrificed at 6, 12, 24, and 48 h, 8 and 15 days after MC withdrawal. The mRNA levels, protein content and enzyme activities of CYP1A1 and 1A2 were determined at different time points. In addition, the binding of MC-AHR-AHR nuclear translocator (ARNT) to the AHREs on the CYP1A1 promoter region was determined by chromatin immunoprecipitation (ChIP) assay in liver and lung tissues. Results: In WT mice, ChIP experiments indicated that transcriptional activation of CYP1A1 was most pronounced at 6 h, followed by 12 h, but declined at later time points. In Cyp1a2_null mice, on the other hand, transcriptional activation of CYP1A1 persisted till 48 h. In lungs, AHR complex binding peak was observed at 12 h and declined later, but Cyp1a2 null mice showed more persistent binding till 48 h with a peak at 24 h. On the other hand, the expression of CYP1A1/1A2 at the mRNA, protein and enzyme levels persisted for up to 48 h both in lung and liver tissues. Conclusions: These results suggest a mechanistic role for CYP1A2 in the molecular regulation of CYP1A1, and that transcriptional activation of CYP1A1 at 6-12 h is sufficient to result in sustained induction of CYP1A mRNA and protein expression for up to 48 h. These studies have important implications for carcinogenesis mediated by PAHs.

3159 CYP1 Substrate Docking and Ligand Access Channels

J. V. Goldstone[3], P. Urban[1], J. Y. Wilson[2], D. Alsop[2], D. Pompon[1] and J. J. Stegeman[3]. [1]*Laboratoire Ingénierie des Systèmes Biologiques et des Procédés UMR INSA/CNRS, Toulouse, France;* [2]*Biology, McMaster University, Hamilton, ON, Canada and* [3]*Biology, Woods Hole Oceanographic Institution, Woods Hole, MA.*

Computational modeling of three-dimensional interactions between cytochromes P450 (CYPs) and their ligands provides a better understanding of their structure-function relationships. Ligand 'docking' provides a prediction of the binding energy and site, which can be related to the probability of enzymatic reaction with that potential substrate. We have examined the computed affinities of an array of more than 100 zebrafish CYP1 substrates for which we have metabolite data, including drugs, steroids, flavonoids, polycyclic aromatic hydrocarbons, and fluorescent substrates. Affinity scores from high throughput docking of compound libraries to zebrafish CYP1 models did not reflect the hit enrichment found in an *in vitro* high throughput screen of 4600 compounds for enzyme turnover. However, computed ligand positions correlate well with observed product ratios for most of the substrates examined in detail. Observed differences between zebrafish CYP1C1, CYP1C2, and CYP1D1 in the regioselectivity for testosterone metabolism were reflected both in the relative positioning of the substrate in the active site, and in the calculated relative affinity values. Positional docking results also showed all PAHs docking in productive orientations, consistent with the results showing that all three expressed enzymes metabolized all these PAHs. The overlay of structures of docked structures gave a view of the most preferred ring of attack proximal to the heme, suggesting a PAH pharmacophore for each enzyme. Overall phylofunctional clustering did not agree with phylogenetic relationships, leading to the identification of amino acids at substrate access channels that may guide substrate selectivity in these cytochrome P450 enyzymes. (NIH P42ES007381 (BU Superfund Center), NIH R01ES015912).

3160 Modulation of Pulmonary Drug Metabolism Enzymes in Cyp1a1/Cyp1a2/Cyp1b1 Triple Knockout Mice

D. Criscoe[2], A. Veith[2], W. Jiang[1], L. Wang[1] and B. Moorthy[1]. [1]*Department of Pediatrics-Newborn, Baylor College of Medicine and Texas Children's Hospital, Houston, TX and* [2]*Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX.*

Rationale: Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental procarcinogens found in particularly high levels in cigarette smoke. PAHs induce the activation of the Cyp1 family of metabolic enzymes (Cyp1a1, Cyp1a2, and Cyp1b1), which play a major role in the bioactivation of PAHs into potent lung carcinogens. Deficiency of these Cyp1 metabolic enzymes may lead to inhibition of carcinogenesis. Hypothesis: The goal of this study was to evaluate the *in vivo* metabolic regulatory response to administration of the PAH 3-methylcholanthrene (3-MC) in the absence of the Cyp1 monooxygenases. Methods: Wild type (C57BL6) mice or Cyp1a1-/Cyp1a2-/Cyp1b1- triple knockout mice were administered 25mg/kg 3-MC i.p. once daily for four days. Lung tissue was harvested from mice sacrificed 1 day, 8 days, 15 days, and 22 days after 3-MC administration, and RT-PCR was performed for several drug metabolism enzymes (Nrf2, Nqo1, Nqo2, and Cpr). Results: In wild type mice, 3-MC induced an immediate 10-fold increase in Nqo1 which returned to baseline over time. In Cyp1 triple knockout mice, 3-MC induced an immediate 5-fold increase in Nqo1 that then returned to baseline more slowly over time, leading to a significant increase in Nqo1 at Day 15 compared to wild type mice. Expression of Cpr was significantly lower in Cyp1 triple knockout mice compared to wild type mice at Day 8. No significant differences were detected for Nrf2 or Nqo2. Conclusions: The fact that Nqo1 induction by 3-MC was attenuated in the Cyp1 triple knockout mice supports the hypothesis that metabolism of 3-MC by Cyp1 enzymes contributes to Nqo1 induction, presumably via an Nrf2-mediated mechanism. The augmented induction of Nqo1 at Day 15 in the triple knockout mice implies that in the absence of the PAH-metabolizing Cyp1 enzymes, clearance of PAHs is hindered, resulting in prolonged PAH-dependent activation of Ahr. Future studies will seek to further elucidate mechanisms.

3161 Cyp1b1 Genotype and PAH Dibenzo[def,p] chrysene Transplacental Exposure: Effects of Wild Type, Null and Transgenic Human CYP1B1 Genotype on Adult On-Set Carcinogenesis in B6129F1 Mice

E. Madeen[3], C. Loehr[3], H. You[3], L. Siddens[3], S. Kreuger[3], F. Gonzalez[1], K. Waters[2] and D. Williams[3]. [1]*National Cancer Institute, Bethesda, MD;* [2]*Pacific Northwest National Laboratory, Richland, WA and* [3]*Toxicology, Oregon State University, Corvallis, OR;* [4]*Vetrinary Medicine, Oregon State University, Corvallis, OR.*

The cytochrome P450 (CYP) 1 family is active toward numerous environmental pollutants, including polycyclic aromatic hydrocarbons (PAHs). Utilizing a mouse model, null for Cyp1b1 and expressing a human CYP1B1 transgene, we tested the hypothesis that hCYP1B1 is important for dibenzo[def,p]chrysene (DBC) transplacental carcinogenesis. Wild-type mCyp1b1, transgenic hCYP1B1(on a mCyp1b1 null background), and mCyp1b1 null mice were assessed, each litter containing a 1:1 ratio of siblings with Ahrb-1/d and Ahrd/d alleles. Pregnant mice were orally gavaged on gestation day 17 with 6.5 or 12 mg/kg of the PAH, DBC, and compared to corn oil controls. The offspring, born on gestation day 19, were evaluated at 10 months of age for mortality, general health, diseases of lymphoid tissues, and lung tumor incidence and multiplicity. Wild type mice were 59% more likely to succumb to T-cell Acute Lymphoblastic Leukemia (T-ALL). The Ahr genotype was not a significant factor in lung tumor incidence, multiplicity, or mortality. Gender was not a significant factor in lung tumor incidence or mortality but males exhibited a significantly greater lung tumor multiplicity. The hCYP1B1 genotype did not impact lung tumor multiplicity. Lung tumor incidence was greater in mCyp1b1 nulls compared to wild-type mice. Transgenic hCYP1B1 mice were more likely to develop lung tumors than Cyp1b1 wild-type but this did not reach statistical significance ($p = 0.07$). Continuing work includes miRNA seq of tumor vs healthy lung tissue from the 10 month time point and neonatal lung tissue, following transplacental DBC exposure.

3162 Fluoranthene (FLA) Is Cardiotoxic and Induces Cytochrome P450 Type 1A (CYP1A) mRNA Expression in *Xenopus laevis* Embryos

M. Sestak, C. Lamoureux, J. Hall, J. Coburn and S. L. Whittemore. *Biology, Keene State College, Keene, NH.* Sponsor: D. Hood.

To extend current understanding of mechanism(s) of toxicity for certain prevalent PAHs, we compared the impact of early PAH exposure on cardiac function and cyp1a mRNA expression using a model system in which sensitivity to AHR agonists is particularly low, providing a useful contrast to results derived from fish studies. Among the several findings revealing considerable variation in developmental sensitivity to PAHs among species, was the observation that FLA, which does not impact normal cardiac function in fish, was cardiotoxic to *Xenopus* embryos. FLA-exposed animals exhibited ventricular tachycardia (0.25-25 μM; 72 hpe), followed by ventricular bradycardia (with atrial tachycardia) and increased incidence of atrioventricular (AV) block at (25 μM; 120 hpe), suggesting that FLA alters both normal cardiac pacemaker and conduction function in *Xenopus*. FLA-induced tachycardia, but not the subsequent bradycardia or AV block, remained evident 48 h after return to control conditions, indicating that pacemaker activity can be altered by earlier limited exposure to FLA. Mortality was increased 8-fold (to 83%) with continuous FLA exposure (25 μM, 7-8 dpe), but no pericardial edema was noted. Although FLA inhibits CYP1A activity in fish, in Xenopus embryos, FLA induced cyp1a(7) mRNA expression 35-fold (25 μM; 72 hpe), suggesting FLA acts as an AHR agonist in that species. In addition, FLA exposure (25 μM; 72 hpe) was associated with a 50% reduction in mRNA levels for the key cardiac transcription factor Tbx5. Collectively, these results suggest that early exposure to FLA alters gene expression and compromises cardiac function in *Xenopus* by interfering with normal pacemaker and conduction activity, effects associated with increased mortality. These findings, along with others not reported here, also reveal a considerable degree of species specificity, between fish and frog, regarding cardiac sensitivity to developmental PAH exposures.

3163 Cytochrome P450 2J2 and Its Potential Protective Role in ROS-Induced Cardiotoxicity

E. A. Evangelista and R. A. Totah. *Medicinal Chemistry, University of Washington, Seattle, WA.*

Cytochrome P450 2J2 (CYP2J2), the major human P450 isozyme expressed in cardiac tissue, is unique among other cytochrome P450 enzymes in that it is a drug metabolizing enzyme with a known endogenous function. CYP2J2 is responsible for oxidizing arachidonic acid to epoxyeicosatrienoic acids (EETs), which are protective in cardiovascular tissue, however, little is known about the regulation of CYP2J2, and thus EET levels, in the heart during drug and disease induced oxidative stress. Previous studies report CYP2J2 to be immune to the canonical inducers of drug-metabolizing CYPs. In this study, an adult primary ventricular cell line was exposed to a variety of stressors that increase reactive oxygen species (ROS) production in the heart. ROS are toxic compounds that can be produced in various diseased states, including hyperglycemia and ischemia-reperfusion, often with serious consequences for the heart. The cells were initially characterized and confirmed to have similar CYP expression as human ventricular heart tissue. Then cells were exposed to treatments that promote oxidative stress and increase endogenous ROS formation including hypoxia and cobalt chloride. When cultured under hypoxic (2.5% oxygen) conditions for 24h, CYP2J2 expression decreased by 50% similar to cobalt chloride (1 mM) treatment for 24h, which decreased expression up to 80%. Further, treating the cells with metformin, a drug shown to reduce endogenous ROS formation, for 72 hours caused an initial >90% decrease in gene expression within the first 24 hours followed by a rapid return to normal expression levels after 48 hours. Finally, cells were treated with increasing concentrations of hydrogen peroxide to mimic ROS insult to the cells. When treated with 0.01% hydrogen peroxide for 24h CYP2J2 expression increased 3-fold. Taken together, these results demonstrate that CYP2J2 and EETs levels are altered when cardiac cells are under oxidative stress and that CYP2J2 may play a protective role against ROS mediated cardiotoxicity.

3164 Establishment of Murine Model for Neurotoxicity of 1-bromopropane: Role of Cytochrome P450

C. Zong[3,4], E. Garner[1], L. Zhang[4], C. Huang[3], X. Zhang[3,4], S. Toyokuni[3], T. Sakurai[4], S. Ichihara[2] and G. Ichihara[4]. *[1]Lovelace Respiratory Reseach Institute, Albuquerque, NM; [2]Mie University, Tsu, Japan; [3]Nagoya University Graduate School of Medicine, Nagoya, Japan and [4]Tokyo University of Science, Noda, Japan.*

Objective: Neurotoxicity of 1-Bromopropane (1-BP) has been reported in both human cases and animal studies. To date, neurotoxicity of 1-BP was reproduced in rats but not in mice due to death induced by hepatotoxicity of 1-BP. The present study aims to establish a murine model for neurotoxicity of 1-BP, through reducing hepatotoxicity by administration of 1-ABT, which is a nonspecific inhibitor to P450s. Methods: First, hepatic and brain CYP2E1 was measured after administration of 1-ABT to mice. Second, the highest exposure level, at which 1-ABT- treated or untreated mice can survive, was determined by a preliminary experiment. Third, 1-ABT-treated or untreated mice were exposed to 1-BP to evaluate hepatotoxicity and biomarker of neurotoxicity. Results: 1-ABT administration inhibited 92–96% of hepatic CYP2E1 activity, but inhibited 62-64% of brain CYP2E1 activity. Preliminary experiments confirmed 1200 ppm as the highest 1-BP exposure level for 1-ABT-pretreated mice, and 250ppm for 1-ABT-untreated mice. The result showed that 1-ABT pretreatment protected mice from 1-BP-induced hepatic histopathological changes such as necrosis, hepatocyte degeneration, and hemorrhage. In mice pretreated with 1-ABT and exposed to 1200 ppm 1-BP, brain weight decreased and hippocampal expression level of Ran protein, a promising biomarker for neurotoxicity identified by a previous study, increased dose-dependently. Conclusions: Controlling P450 activity enables us to establish murine model for neurotoxicity of 1-BP.

3165 Transcriptional Upregulation of Cyp1a1 and Cyp1b1 by Alkylated Polycyclic Aromatic Hydrocarbons Found in Crude Oil in the Human RPTEC/TERT1 Cell Line

B. Simon-Friedt[1], M. Wilson[1], E. Overton[2], P. Adhikari[2] and J. Wickliffe[1]. *[1]Global Environmental Health Sciences, Tulane University, New Orleans, LA and [2]School of the Coast and Environment, Louisiana State University, Baton Rouge, LA.*

Polycyclic aromatic hydrocarbons (PAHs) are chemicals that are abundant in the environment, can be generated through the process of incomplete combustion, and are relatively common hydrocarbons in crude oil. Some PAHs are known to be hazardous to human health due to their environmental persistence and carcinogenic properties. Although there are many types of PAHs, individual PAHs may produce different biochemical and toxicological responses in biological systems depending on their chemical structures. Many regulatory assessments are based on a few, unsubstituted PAHs that have been widely used for toxicity testing, but there has been comparatively little research on substituted PAHs such as those with one or more alkyl side groups. In order to determine if alkylated PAHs elicited different transcriptional responses compared to their unsubstituted parent compounds, we exposed a metabolically competent human renal cell line (RPTEC/TERT1) to specific PAHs found in MC252 oil. We found that 4- and 6-methylchrysene as well as 6,12-dimethylchrysene induce CYP1A1 and CYP1B1 gene expression significantly more than unsubstituted chrysene. We also found that 10-methylbenzo[a]pyrene induces CYP1A1 and CYP1B1 more strongly than benzo[a]pyrene. These results indicate that future studies and regulatory testing should consider including substituted PAHs and mixtures of PAHs in toxicity assessments.

3166 Analysis of Micrornas Expression Profiles to Identify Specific Toxicity Signatures of Liver Injury for Drugs Sharing Similar Structure

Y. Wang, B. Gong, Z. Liu and W. Tong. *National Center for Toxicological Research, Jefferson, AR.* Sponsor: Y. Wang.

Amiodarone (AMI) and benzbromarone (BBR) are structurally similar, but exhibits different severity of liver injury in humans which could not be distinguished with the current approaches applied in preclinical toxicity assessment. MiRNAs have been reported to associate with disease biological processes, including drug induced liver injury (DILI). Understanding the mechanistic roles of miRNAs could add value in prediction of DILI at the earlier stage of drug discovery and development. Aim to this, we generated miRNAs expression data with next generation sequencing technology from rat livers treated with AMI and BBR at multiple doses and time-points. Compared the expression patterns of miRNAs between AMI and BBR, six directionally opposite differentially expressed miRNAs (DEMs) identified at high dose and all of six were detected earlier after treatment of BBR than AMI. Specifically, 18 DEMs were detected at one of two drugs or deregulated inversely between AMI and BBR. Further annotation with public databases from GEO demonstrated that all of these DEMs are mechanistically involved in DILI through regulation of their target genes. In addition, miRNA-29 and miRNAs-155 were deregulated after treatment of AMI while not changed in BBR. Literature searching indicated that both miR-29 and miR-155 have been reported to play roles in preventing the progress of liver injury. This may partially explain the difference in severity of liver injury caused by AMI and BBR. We concluded that miRNAs expression analysis may provide insightful and add-on value to the current preclinical approaches to enhance the capability in detection of DILI at the earlier stage of drug discovery and development.

3167 Open Dump Exposure Destroys Liver of Wistar Rats

P. U. Nwoha[1], G. E. Waritimi[3], F. Onyije[3], A. Antoni[3] and M. O. Ijomone[2]. *[1]Anatomy and Cell Biology, Obafemi Awolowo University, Ife, Nigeria; [2]Human Anatomy, Cross River University of Technology, Okuku, Nigeria and [3]Human Anatomy, Niger Delta University, Amassoma, Nigeria.* Sponsor: B. Gadagbui.

Open waste dump sites emit dangerous gasses and chemicals that are injurious to the body. Yet these sites are commonly found in towns and villages in most third world countries, including Nigeria, where they can pose serious health problems to residents, workers and scavengers. The organs of the body, particularly the large metabolic organ, the liver, may be compromised in this situation, hence the need for this study. Young adult male Wistar rats, approximate age 70 days, were used for this study. Experimental rats were housed in a hut inside a large refuse dump site located along Amassoma Road, outskirts of Yenagoa, Bayelsa state, Nigeria. Control rats were kept in the Animal Holding of the Department of Human Anatomy, Niger Delta University, Amassoma, Bayelsa State. Animals in both groups were housed in clean cages for eight months, with adequate food and water provided. At the end of the period, the animals were sacrificed by ether anaesthesisa, blood taken for biochemical analysis, and liver specimen for H & E histology. Results showed abnormal swellings and numerous yellowish fat deposits in the liver of the exposed but not control rats. Significant increase in the levels of liver marker enzymes (ALP, AST, ALT) in the sera of experimental rats were noted compared to the control ($P<0.001$). There was significant decrease in serum albumin, and total protein of the experimental compared to the control rats ($P<0.05$). Histological studies with H&E showed severe hepatocellular necrosis and degeneration of the liver of the experimental rats. These observations suggest that exposure of rats to refuse dump is very destructive of liver tissue and functions.

3168 A Comprehensive Annotation for the Risk of Drug-Induced Liver Injury in Humans Based on the US FDA-Approved Drugs Labeling

M. Chen[2], A. Suzuki[1], S. Thakkar[2], Y. Ke[2], C. Hu[2] and W. Tong[2]. *[1]Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR and [2]National Center for Toxicological Research, US FDA, Jefferson, AR.*

Drug-induced liver injury (DILI) is one of the major safety concerns for drug developers, regulators and clinicians. Assessing a drug's likelihood to cause DILI with the current preclinical models is not very successful, thus active research has been placed in developing new tools and approaches to better understand and predict DILI. These efforts require a reference dataset containing a comprehensive set of drugs accurately annotated with their DILI risk in humans. We previously developed a DILI annotation schema based on information from the FDA-approved drug labeling and generated a benchmark dataset which contained 287 drugs that are grouped into three levels of DILI severity categories. In this study we further improved the method by incorporating adjudicated culprit drugs and the analysis was done for the entire drug list reported in the FDA drug labelling. The analysis yielded the largest dataset with DILI annotation data in the public domain, which contains 1037 drugs approved by the FDA before the year of 2010. Specifically, 356 drugs that were adjudicated as culprit drugs in three large case reports sources (i.e. NIH LiverTox database, DILIN studies, and Suzuki et al. 2010) and some additional drugs from literature search were collected to support the new schema. Moreover, we analyzed the therapeutic landscape of the reference dataset, and the top 10 therapeutic groups of drugs with DILI concern were antineoplastic (N=22), psychoanaleptics (N=14), antivirals (N=12), antibiotics (N=9), nonsteroidal anti-inflammatory drugs (NSAIDs) (N=9), immunostimulants (N=6), immunosuppressants (N=6), antimycotics (N=5), antiepileptics (N=5) and cardiac therapy (N=5). We also re-evaluated a published "rule-of-two" model (Chen et

al., Hepatology, 2013), and found that the assessed performance using this reference dataset is consistent with the previous publication. The reported reference dataset will not only contribute to model development for the early identification of drug candidates associated with serious DILI risk, but will also aid in the clinical diagnosis when considering drugs that are suspected to be the cause of hepatotoxicity.

3169 Fluorouracil Sensitizes Hepatocytes to Ischemia/Reperfusion Injury

K. Go, S. Lee, R. U, I. Zendejas, K. Behrns and J.-S. Kim. *Surgery, University of Florida, Gainesville, FL*. Sponsor: J.-S. Kim.

Introduction: Fluorouracil (5-FU) is a chemotherapy agent that inhibits thymidylate synthetase and is catabolized primarily in the liver. While this pyrimidine analogue is a mainstay of treatment against multiple solid tumors, several studies have reported its potential association with hepatotoxicity. We investigated the effects of 5-FU on hepatocytes. Methods: To examine acute effects, hepatocytes isolated from C57 BL/6 male mice were treated with 2 μM of 5-FU for 12, 24, and 36 hours. To investigate chronic effects of this agent, saline or 20 mg/kg of 5-FU were injected into mice twice weekly for 4 weeks prior to hepatocyte isolation. Cells were subjected to 2 hours of ischemia followed by reperfusion, a mild stress that induces minimal injury in normal hepatocytes. Autophagy flux was measured by immunoblotting of the microtubule-associated protein light chain 3 (LC3) in the presence or absence of chloroquine. Cell death was determined by propidium iodide (PI) fluorometry. Changes in lipid droplet (LD), mitochondrial membrane potential , onset of mitochondrial permeability (MPT) and mitochondrial morphology were assessed by confocal microscopy with nile red, tetramethylrhodamine methylester and calcein. Results: Acute exposure to 5-FU increased the number of LD and size of LD but not to a significant level. While 5-FU did not induce cell death under normoxic conditions, exposure of 5-FU-treated cells to ischemia/reperfusion (I/R) induced MPT onset, loss of membrane potential and cell death, suggesting mitochondrial dysfunction. In contrast to vehicle controls, hepatocytes from mice chronically exposed to 5-FU showed a substantial increase in necrosis after I/R. Less autophagy flux was observed in these 5-FU-treated cells. Mitochondrial morphology in chronically exposed cells was highly elongated compared to vehicle controls. Conclusions: Both acute and chronic exposure to 5-FU cause mitochondrial alterations and increased sensitivity to I/R injury. Since the mitochondria are key players in I/R injury, mitochondrial dysfunction may be a mechanism underlying 5-FU-mediated hepatotoxicity.

3170 Steatohepatitis Biomarkers Reveal Elevated Liver Disease in Polychlorinated Biphenyl-Exposed Cohort

H. B. Clair[4], C. Pinkston[4], G. Brock[4], M. Pavuk[1], N. Dutton[1,2], K. C. Falkner[4], R. A. Prough[4] and M. Cave[3,4]. [1]*Agency for Toxic Substances Disease Registry, Atlanta, GA;* [2]*ORISE Research Participation Program, Atlanta, GA;* [3]*Robley Rex Veterans Affairs Medical Center, Louisville, KY and* [4]*University of Louisville, Louisville, KY*.

Purpose: Anniston, Alabama residents were historically exposed to high levels of PCB-containing waste in the environment around the former Monsanto plant. We earlier reported PCB levels 2-3 fold higher than the general US population and elevated serum liver disease mechanistic biomarkers in this population. The prevalence of liver injury indicated by Cytokeratin-18 (CK18) was 60%. The injury appeared to be primarily of necrotic character, consistent with toxicant-associated fatty liver disease (TAFLD). A resampling of this population in 2014 was undertaken to assess exposure and disease changes. Methods: Serum samples were obtained from 352 Anniston, AL residents (ACHS2) originally screened in 2005-2007 (ACHS1). Whole (CK18-M65) and caspase-cleaved CK18 (CK18-M30), and serum levels of cytokines and adipokines were analyzed. Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and direct/indirect bilirubin were also measured. Results: 62% of ACHS2 study participants had CK18 elevations, increased from 60% in ACHS1. In both ACHS1 and ACHS2, a pattern of elevated CK18-M65 with normal CK18-M30 was accompanied by serum pro-inflammatory cytokine elevations, metabolic dysfunction and fibrosis. Serum transaminase abnormalities were found in only 10% of participants, mainly in those with a non-TASH CK18 elevation. Conclusions: The strikingly high and sustained prevalence of liver disease in ACHS2 indicated by CK18 is not reflected by concurrent elevations in serum transaminases consistent with our previous findings in industrial workers exposed to PVC. We suggest that current clinical laboratory biomarkers (ALT/AST) may underestimate liver injury in populations exposed to specific environmental chemicals, including PCBs.

3171 Repeated Administration of Atovastatin Alters the Expression of Genes Related to Inflammation, Bile Acid Metabolism and Circadian Clock in Livers of Mice

W.-K. Li[2], Y.-J. Xu[2], Y.-F. Lu[2], Z.-D. Fu[1] and J. Liu[2]. [1]*University of Washington, Seattle, WA and* [2]*Zunyi Medical College, Zunyi, China*.

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors clinically used for hyperlipidemia. Statins are generally safe but could induce cholestasis. The present study aimed to examine the effects of atorvastatin at high doses on hepatic gene expression related to inflammation, bile acid metabolism and the expression of circadian clock genes in mice. Adult male mice were given atovastatin (10, 30, and 100 mg/kg, po) daily for 30 days, and blood biochemistry, histopathology, and gene expressions were examined. Repeated administration of Atovastatin did not affect animal body weight gain and liver weights. Serum enzyme activities were in the normal range. Histologically, the high dose of atovastatin produced spotted swollen hepatocytes, foci of the feathering-like degeneration and inflammatory cell infiltration, together with increased expression of early growth response-1 (Egr-1) and metallothionein-1 (MT-1). Atovastatin increased the expression of Cyp7a1, along with farnesoid X receptor (FXR) and small heterodimer partner (SHP). In contract, atovastatin decreased the expression of bile acid transporters such as sodium-taurocholate cotransporting polypeptide (Ntcp) and bile salt export pump (Bsep), Ostα, and Ostβ. The most dramatic change was the 30-fold induction of Cyp7a1. Since Cyp7a1 is a circadian clock-controlled gene, we further examined the effect of atovastatin on clock gene expression. Atovastatin increased brain and muscle Arnt-like protein-1 (Bmal1) and neuronal PAS domain protein 2 (Npas2) in a trend similar to Cyp7a1 increases; Atovastatin decreased period circadian clock 2 (Per2) and D site of albumin promoter (Dbp) expression, while it had no effects on cryptochrome circadian clock 1 (Cry1) and Rev-erba expression. Thus, repeated administration of atovastatin at high doses could affect bile acid metabolism and disposition. Atovastatin markedly increased the expression of the bile acid synthesis rate-limiting enzyme Cyp7a1, together with alterations in the expression of circadian clock genes.

3172 Use of LC/MS/MS Proteomic Biomarkers for Early Detection of Acetaminophen-Induced Liver Toxicity in Preclinical Pharmaceutical Toxicology Assessment

A. Licollari and Y. Sydorskyy. *Toxicology, Nucro-Technics, Scarborough, ON, Canada*.

Introduction: The purpose of this study was to develop a LC/MS/MS method for the quantification of biomarkers in the serum of rats to predict potential liver toxicity and monitor clinical safety. A panel of protein biomarkers was selected based on a thorough review of scientific publications. Acetaminophen (APAP) as a known hepatotoxicant was used to provide rapid toxicological assessment. Methods: Two groups of Sprague-Dawley rats were dosed orally either with a high single dose of acetaminophen or Water for Injection. Approximately 24 hours after treatment, each animal was anaesthetized by exposure to isoflurane, blood samples were collected from the abdominal aorta and were used for standard clinical pathology investigations (hematology and serum biochemistry parameters) and serum was collected for biomarker quantification. An LC/MS/MS method was developed for quantification of 83 proteomic biomarker candidates in the serum of treated rats. Classical toxicology analysis and biomarker quantification were performed in animals treated with APAP and Water for Injection. Subsequent biomarker data were submitted for a clustering analysis. Results: 9 of 83 safety biomarkers evaluated by the above methods were determined to be predictive of APAP hepatotoxicity. The cluster analysis demonstrated that biomarkers accurately segregated the rats dosed with APAP and Water for Injection in two distinguished clusters. Conclusions: The LC/MS/MS method used to quantify biomarkers for the detection of early APAP toxicity in rats adequately discriminated toxic and non-toxic treatment with significant enhancement over current standard clinical pathology evaluation. The sensitivity and specificity of hepatotoxicity detection needs to be re-evaluated with more physiologically-relevant doses. Once fully validated, this test is expected to be readily transferable to a clinical setting and has a potential to become a valuable tool for toxicological assessment of liver toxicity.

3173 Induction of Drug-Induced Liver Injury (DILI)-Associated Genes by Hepatotoxic Drugs in Keratinocytes

R. Fujiwara, R. Hirashima and T. Itoh. *School of Pharmacy, Kitasato University, Tokyo, Japan.*

Drug-induced liver injury (DILI) is one of the most common adverse drug reactions (ADRs) and the leading reason for the withdrawal of approved medications from the market. *In vitro* models and pre-clinical animal models have been developed to screen compounds with high risk for DILI; however, a number of DILI cases have still been reported. To avoid the development of DILI, identification of the individuals who can possibly develop DILI needs to be developed. It was recently reported that expression of S100A7, a DILI-associated gene, was increased in the enalapril-treated human keratinocytes, indicating that keratinocytes and hepatocytes might share a similar responsiveness to drugs. To further investigate the responsiveness to drugs in the keratinocytes, in the present study, human and mouse keratinocytes were treated with hepatotoxic drugs. We determined the expression level of DILI-associated genes in the control- and drug-treated cells. In this study, human keratinocytes (HaCaT cells) and mouse primary keratinocytes were treated with hepatotoxic drugs such as diclofenac, flutamide, ketoconazole, and pioglitazone. Expressions of DILI-associated genes such as S100A8, S100A9, NALP3, IL-1β, and RAGE were quantified by Q-PCR. The mRNA expression level of IL-1β was increased by 3-fold in the HaCaT cells treated with flutamide. NALP3 mRNA expression level was increased by 3-fold in mouse keratinocytes after flutamide exposure. Other hepatotoxic drugs also induced the DILI-associated genes in human and mouse keratinocytes. These results indicate that keratinocytes and hepatocytes share a similar responsiveness to hepatotoxic drugs. It has been reported that LPS enhanced the liver damage produced by hepatotoxic drugs. We found that NALP3 expression was higher in the keratinocytes isolated from LPS-injected mice compared to that in the control mouse keratinocytes. Furthermore, flutamide significantly induced the NALP3 expression in the keratinocytes isolated from LPS-injected mice. It was shown that there was a wide inter-individual variability in the expression and inducibility of IL-1ß in the mouse keratinocytes. These findings suggest that the higher expression and inducibility of the DILI-associated genes in keratinocytes can be predictive markers of DILI in individuals.

3174 Inhibition of PARP Activity Reduces Drug-induced Toxicity

K. Haire, J. Coyle, K. Harand, J. McCluskey, G. Johnson, M. Bourgeois and R. Harbison. *Environmental Occupational Health, College of Public Health, Tampa, FL.*

Drug toxicity may cause liver injury, resulting in damage to hepatocytes and organ function. This cytotoxic event may be initiated by enhancing poly (ADP-ribose) polymerase (PARP) activity. The purpose of this study was to evaluate the role of PARP in drug-induced toxicity. Cocaine and acetaminophen-induced hepatotoxicity were used to evaluate the efficacy of PARP inhibitors to alter the drug-induced hepatotoxicity. A PARP inhibitor, 1,5-dihydroxyisoquinoline (DIQ), was used to elucidate the role of PARP in drug-induced hepatotoxicity. A 50 mg/kg dosage of cocaine or a 300 mg/kg dosage of acetaminophen (APAP) was administered, followed by a 10 mg/kg dosage of DIQ either concurrently or one hour following drug treatment. PARP activity, DNA fragmentation, and glutathione (GSH) levels were measured in liver tissue. Cocaine and APAP effects were evaluated at 6 and 18 hours following treatment when the highest levels of PARP activity were observed. PARP activity was evaluated and correlated with alanine transaminase (ALT) levels following DIQ treatments. DIQ administered concurrently with drug treatment significantly decreased ALT levels indicating a protective effect. DIQ blocked elevation of ALT activity for both cocaine and APAP. DIQ also significantly reduced PARP activation. Drug treatment resulted in activation of PARP activity from 0.2 to 1.3 IU/20 ug protein for cocaine and 0.26 to 1.5 IU/20ug protein for APAP. DIQ treatment reduced this activation to about 0.5 to 0.7 IU/20 ug protein. Decreased PARP activity was observed following DIQ treatment with both concurrent drug treatment and treatment following drug administration. Cocaine and APAP treatment did not cause DNA fragmentation. GSH levels did not correlate with DIQ alteration of PARP activity. The mechanism of DIQ effects on drug-induced hepatotoxicity appears to be GSH independent. DIQ was effective in reducing drug-induced hepatotoxicity and preserving organ function.

3175 miR-122 Release in Exosomes Accompanies Tolvaptan-Induced Mitochondrial Dysfunction, Oxidative Stress and Apoptosis in a Micropatterned Primary Human Hepatocyte Coculture Model

M. Mosedale[2], J. S. Eaddy[2], O. J. Trask[2], N. S. Holman[2,3], K. K. Wolf[2], E. LeCluyse[2], W. J. Brock[1], S. E. Roth[1] and P. B. Watkins[2,3]. [1]*Otsuka PDC, Inc., Rockville, MD;* [2]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [3]*The University of North Carolina at Chapel Hill, Chapel Hill, NC.*

The vasopressin receptor 2 antagonist tolvaptan has been associated with idiosyncratic drug-induced liver injury in polycystic kidney disease patients. To gain insight into the possible mechanism(s) underlying tolvaptan hepatotoxicity, the HepatoPac® platform was used to evaluate the activation of stress response pathways in primary human hepatocytes using a combined experimental approach of high-content imaging, toxicogenomics, and exosome analysis. Hepatocytes from 3 adult donors were exposed to tolvaptan (0.01 - 50 μM) or a DMSO control continuously for 4, 24, or 72 h. Hepatocyte cellular imaging revealed a significant effect of tolvaptan concentration on programmed cell death ($p<0.05$) that was associated with mitochondrial dysfunction and cytochrome c release ($p<0.05$ at 50 μM). Microarray analysis revealed statistically significant gene expression changes (false discovery rate $p<0.05$ and fold-change>|1.5|) associated with the activation of Nrf2-mediated oxidative stress and unfolded protein response pathways. While ALT release was not significantly affected, urea production decreased dose-dependently ($p<0.05$ at 20 and 50 μM). A trend toward increased microRNA-122 (miR-122) release into the medium also was observed, but differences were significant only in the exosomal fraction isolated using ExoQuick-TC ($p<0.05$ at 50 μM). We conclude that mitochondrial dysfunction, oxidative stress, and apoptosis may contribute to tolvaptan-induced liver injury. Furthermore, increases in exosomal miR-122 release may be important mechanistically as exosomal miR-122 has been shown recently to promote Kupffer cell activation. Ongoing studies will investigate circulating miR-122 as a biomarker to manage the risk of tolvaptan hepatotoxicity in the clinic.

3176 Elevation of Serum Toxicity Biomarkers by Reduced Protein Clearance in the Absence of Organ Injury

A. Wolf and F. Pognan. *Preclinical Safety, Novartis Institute for Biomedical Research, Basel, Switzerland.*

Serum biomarkers are useful tools to monitor drug safety in preclinical and clinical studies, however sometimes toxicity biomarker are not matching the real drug-induced organ injuries. False interpretations of increased alanine aminotransferase (ALT) and creatine kinase (CK) occur frequently in animals after treatment with compounds affecting the proliferation and differentiation of Kupffer cells (KCs) by inhibiting the macrophage colony-stimulating factor 1 (CSF1) pathway. There is indirect evidence that the inactivation of KCs can lead to increased half-life of these proteins and thus elevating their base line values, which can be wrongly indicating target organ toxicity. It was the purpose of the present studies to explored the role of KCs by measuring the clearances of intravenously injected histidine (His)-labeled recombinant enzymes ALT-1 (isoform of ALT) in rats treated with the CSF-1 receptor antagonist BLZ945 and that of His-labeled CKM (CK-MM isoform) in monkeys treated by the CSF1 binding mono-clonal antibody MCS110. The BLZ945 and MCS110 treatments resulted in elevations of the endogenous serum ALT, AST or CK without causing histological lesions or increasing target organ specific miRNAs. Inversely both compounds were leading to a pharmacological reduction of KCs or pharmaco-dynamic (PD) effects. The elimination kinetics of the intra venous injected His-tagged ALT1 and CKM were analyzed by non-compartmental (NCA) and a population analysis. Both analyses consistently demonstrated significantly increased of the elimination half-lives and delayed clearance rates of the His-tagged proteins after the BLZ945 and MCS110 treatment. The present data strongly suggest that reduced protein clearances by KCs can increase the basal activities of serum enzymes in rats and monkeys, which is disconnected from drug-induced liver or muscle damages.

3177 Relationship of AhR Genotype to DE-71 Tumor Formation in Wistar Han Rats

B. A. Merrick[2], T. Maynor[1], A. E. Brix[1], G. E. Kissling[2], M. J. DeVito[2], S. Waidyanatha[2] and J. K. Dunnick[2]. *[1]ILS, Inc., Research Triangle Park, NC and [2]Division, National Toxicology Program, NIEHS, Research Triangle Park, NC.*

DE-71 (Technical Grade) is a flame retardant, pentabromodiphenylether (PBDE) mixture. A 2-year bioassay of DE-71 in Wistar-Han rats at 50 mg/kg, p.o. had a higher incidence of hepatocellular adenomas, carcinomas and cholangiomas compared to corn oil controls suggesting PBDEs were hepatocarcinogenic. However, the DE-71 test article has low levels of polybrominated dibenzodioxins and furans (~7x10-6 %) such that AhR activation might contribute to malignancy. A confounding factor is an exon 10 boundary mutation in AhR occurs with varying frequency in the Wistar Han strain, potentially interfering with AhR function and leading to resistance in tumor formation. Thus, we evaluated the relationship of AhR genotype to liver tumor formation in DE71 treated rats. We tested the hypothesis that wild type 'wt' Ahr genotype rats (G/G) would have a higher incidence of liver tumors compared to mutant 'mt' AhR genotype rats, as mt AhR homozygous (A/A) or mt AhR heterozygous (G/A). Two-year DE71 exposed female rats were selected for genotyping since their liver tumor incidence was twice that of males. DNA was extracted from liver paraffin sections and from kidney sections (a non-target tissue) for 60 control and 58 exposed rats. AhR genotyping was performed with a custom TaqMan® SNP Genotyping assay and/or Sanger sequencing. Results showed that of the 118 liver samples, 26 were wt G/G at 22%, 51 were G/A at 43% and 39 were A/A at 33%. Genotypes of only 2 (2%) rats were undetermined. Statistical comparisons tested relationships of AhR genotype to liver tumor type but no significant differences were found even when various liver tumor types were combined such as adenomas and carcinomas or single and multiple tumors. The distribution of AhR genotypes in female Wistar Han rats suggested that DE-71-mediated liver tumor formation was independent of a fully functional AhR receptor since over three-fourths of the Wistar rats carried a mt AhR allele. Alternatively, the level of AhR activation may have been inadequate to contribute to tumor formation due to low AhR affinity of PBDE in DE-71 during chronic exposure or because of negligible amounts of dioxin-like contaminants in DE-71. In summary, genotyping of female Wistar Han rats for an AhR mutation from paraffin archival samples did not show an association with incidence of liver tumors in the 2-year chronic exposure studies of DE-71.

3178 The Use of Circulating Plasma Biomarkers to Study Mechanisms of Liver Injury Following Drug Induced Ischemic Hepatitis

J. L. Weemhoff[3], B. L. Woolbright[3], M. R. McGill[3], S. C. Curry[1], D. J. Antoine[2] and H. Jaeschke[3]. *[1]Department of Medicine, University of Arizona Banner Good Samaritan Medical Center, Phoenix, AZ; [2]Department of Molecular and Clinical Pharmacology, University of Liverpool MRC Centre for Drug Safety Science, Liverpool, United Kingdom and [3]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Hypoxic hepatitis is a clinical condition precipitated by prolonged periods of oxygen deprivation to the liver and can have several underlying causes. Despite its prevalence in critically ill patients, which can reach upwards of 10%, very little is known about the mechanisms of injury. Thus, we set out to measure previously identified circulating biomarkers in an attempt to describe mechanisms of injury following drug induced ischemic hepatitis (DIIH). Plasma from patients diagnosed with DIIH was collected for this study and biomarkers of hepatocellular injury, mitochondrial damage, and cell death were measured. These results were compared against results obtained from the well characterized acetaminophen toxicity model. At peak injury, ALT measured 4082±606 U/L and gradually decreased over 5 days, corresponding to the clinically observed pattern of DIIH. Peak levels of GDH were significantly higher in these patients than in acetaminophen patients (1579±299 vs. 527±117 U/L). Plasma levels of DNA fragments mimicked hepatocellular injury as measured by ALT and miRNA-122. Interestingly, we found a significant increase in caspase-cleaved cytokeratin-18 however the full-length form greatly exceeded the cleaved form at the time of maximum injury (45837±12085 vs. 2528±1074 U/L). We also found an increase in acHMGB1 at later time points indicating a possible role of inflammation. We conclude that the mechanisms of injury following DIIH involves mitochondrial damage and DNA fragmentation. Importantly, necrosis, rather than apoptosis, is the main mode of cell death.

3179 Nonalcoholic Steatohepatitis Alters Cytochrome P450 Activity In Pediatric Patients

H. Li[3], M. J. Canet[3], J. D. Clarke[3], J. Han[3], S. A. Xanthakos[2], J. E. Lavine[1], R. P. Erickson[3] and N. J. Cherrington[3]. *[1]Columbia University, New York, NY; [2]University of Cincinnati, Cincinnati, OH and [3]Pediatrics, University of Arizona, Tucson, AZ; [4]Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Variable drug responses in pediatric patients are often dependent on inter-individual variations in the activity of drug-metabolizing enzymes, like cytochrome P450s (CYPs). Nonalcoholic steatohepatitis (NASH) is the most severe form of what has become the most common chronic liver disease in adolescents. Additionally, NASH has been identified as a source of significant inter-individual variation in drug metabolism. Alterations of hepatic CYP activity were observed in an *ex vivo* analysis of adult human NASH livers and *in vivo* in a rodent model of NASH. The purpose of this study was to investigate the impact of NASH on the major CYP activities in pediatric NASH patients. All recruited healthy and NASH pediatric subjects received a cocktail of four probe drugs: caffeine (CYP1A2, 100mg), omeprazole (CYP2C19, 20mg), losartan (CYP2C9, 25mg), and midazolam (CYP3A4, 2mg). Venous blood samples were collected pre-administration and 1, 2, 4 and 6 hours post-administration. The drugs were quantified in plasma samples via LC-MS/MS. The significant increase of the area under the curve (AUC) value for omeprazole and decrease of metabolism AUC ratio for metabolite to parent omeprazole in pediatric NASH subjects indicate a decrease of CYP2C19 activity. The decreased AUC value of 1-hydroxy-midazolam, the major metabolite of midazolam, and decreased AUC ratio of metabolite to parent midazolam in pediatric NASH subjects suggest a loss of CYP3A4 activity. Overall, partially consistent with our *ex vivo* data in adult human, pediatric NASH leads to significant decreases in CYP2C19 and CYP3A4 activity, but no change in CYP2C9, which is distinct from CYP2C9 induction in adult NASH. Therefore, NASH could lead to unique variable drug responses in pediatric patients, and should be considered in pediatric drug dosage determinations.

3180 Perfluorinated Compounds Activate Nrf2 via Inhibiting Autophagy in Human and Mouse Liver/Hepatoma Cells

Y. Le and X. Cheng. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Epidemiological studies have shown that higher serum level of perfluorinated compounds (PFCs) is positively associated with higher risk of liver disorder, hyperlipidemia, and neurotoxicity in humans. We previously reported that PFCs activated nuclear factor erythroid 2-related factor (Nrf) 2 in mouse liver and cultured hepatoma cells. Nrf2 is differentially activated by oxidative stress and autophagy repression. So far, the underlying mechanism responsible for activation of Nrf2 by PFCs remains largely unknown. This study was designed to determine whether PFCs repress autophagy and thus activate Nrf2. In our studies, as expected, we first showed that PFCs dose-dependently increased heme oxygenase (HO)-1 and NAD(P)H-quinone oxidoreductase (Nqo)-1 mRNA expression in mouse liver as well as in human Hep3B hepatoma cells. We further showed that a single intraperitoneal administration of perfluorononanoic acid (PFNA) (0.1mmol/kg) for 4 and 14 days decreased microtubule-associated protein 1 light chain 3 beta (LC3B)-I/LC3B-II conversion in mouse liver, indicating that autophagy was repressed by PFNA. In addition, in mouse liver and cultured human Hep3B hepatoma cells, PFNA treatment increased p62 protein level in the cytosol, indicating that PFNA administration leads to p62 accumulation in the cytosol. In summary, this study demonstrated that PFCs activate Nrf2, partially mediated by PFC-induced autophagy suppression and subsequent cytosolic accumulation of p62 protein.

3181 Temporal and Dose-Dependent Increases in Exosomal RNA Precede Hepatocellular Toxicity in Human and Rodent Models of Drug-Induced Liver Injury

N. S. Holman[1,2], M. Mosedale[1], K. K. Wolf[1], J. S. Eaddy[1], P. B. Watkins[1,2] and E. L. LeCluyse[1,2]. *[1]The Hamner-UNC Institute for Drug Safety Sciences, Research Triangle Park, NC and [2]University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Alterations in the abundance and composition of circulating liver-specific RNAs have been observed in human and rodent models of drug-induced liver injury (DILI). These RNAs remain stable in biofluids largely due to encapsulation within extracellular vesicles such as exosomes. Hepatocyte-derived exosomes (HDE) have been shown to modulate the

phenotype of recipient cells, including Kupffer cells and cholangiocytes. Therefore, we hypothesize that HDE regulate an individual's response to DILI by signaling to other cell types, which likely involves changes in HDE content prior to overt injury. To examine drug-induced alterations in HDE, the present work utilized the prototypical hepatotoxicant acetaminophen (APAP) to explore the time- and dose-dependent dynamics of HDE release. Exosomes were isolated using ExoQuick precipitation reagent. An *in vivo* study was conducted in male Sprague-Dawley (SD) rats administered APAP (0, 500 or 1400 mg/kg) by gavage (n=6). Plasma from the 500 mg/kg group revealed significant elevations in exosomal albumin mRNA ($p<0.05$) at 24 hr post-dose in the absence of increased plasma ALT. Exosomal and protein-bound microRNA-122 (miR-122) also increased, but did not reach statistical significance until hepatotoxicity was observed. To determine the fidelity of primary rat hepatocyte cultures to recapitulate these events, hepatocytes isolated from male SD rats (n=3) were exposed to a concentration range of APAP (0 - 30 mM) for 24 hr. Consistent with our *in vivo* findings, exosomes isolated from culture medium demonstrated significant albumin mRNA elevations at APAP concentrations as low as 1.1 mM ($p<0.05$), while ATP loss and LDH leakage were not significantly altered until 30 mM. The human relevance of these data was examined using primary human hepatocytes (n=7 donors) exposed to a sub-toxic APAP concentration (10 mM) for 24 hr. In the absence of overt necrosis, APAP exposure produced changes in exosomal albumin mRNA, and caused statistically significant elevations in exosomal miR-122 across all donors ($p<0.001$). We conclude that significant alterations in the liver-specific RNA content of HDE occur prior to hepatocellular injury, a finding that translates across *in vivo* and *in vitro* experimental platforms as well as across species.

PS 3182 Enhancement of Acetaminophen-Induced Chronic Hepatotoxicity in Restricted Fed Rats: A Nonclinical Approach to Acetaminophen-Induced Chronic Hepatotoxicity in Susceptible Patients

K. Kondo, N. Yamada, Y. Suzuki, K. Toyoda, T. Hashimoto, A. Takahashi, A. Kobayashi, T. Shoda, H. Kuno and S. Sugai. *Toxicology Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., Hadano, Kanagawa, Japan.*

Acetaminophen (APAP) is a recognized and widely used analgesic and antipyretic medication. However, some patients encounter liver injury after repeated APAP dosing at therapeutic doses. In the present study, we focused on the nutritional state as one of the risk factors of APAP-induced chronic hepatotoxicity in humans and investigated the contribution of undernourishment to susceptibility to APAP-induced chronic hepatotoxicity using an animal model mimicking undernourished patients. Rats were divided into 2 groups: the ad libitum fed (ALF) and the restricted fed (RF) rats and were assigned to 3 groups (n=8/group) for each feeding condition. The animals were given APAP at 0, 300 and 500 mg/kg for 14 weeks under each feeding condition. Plasma and urinary glutathione-related metabolites and liver function parameters were measured during the dosing period and hepatic glutathione levels were determined at the end of the dosing period. In the APAP-treated ALF rats hepatic glutathione levels were increased and hepatic function parameters were not changed, but in the APAP-treated RF rats hepatic glutathione levels were decreased at 500 mg/kg and hepatic function parameters were increased at 300 and 500 mg/kg. Moreover the urinary endogenous metabolite profile after long-term treatment with APAP in the ALF and RF rats was similar to that in human non-responders and responders to APAP-induced chronic hepatotoxicity, respectively. In conclusion, the RF rats were more sensitito APAP-induced chronic hepatotoxicity than the ALF rats and were considered to be a useful model to estimate the contribution of the nutritional state of patients to APAP-induced chronic hepatotoxicity.

PS 3183 Co-Occurrence of Genetic Variations and Drug Properties Is Associated with Increased Risk of Drug-Induced Liver Injury in Humans

J. M. Ding[1,2], M. Chen[2], Z. Liu[2], H. Fang[2] and W. Tong[2]. [1]*College of Natural Sciences, The University of Texas at Austin, Austin, TX and* [2]*Division of Bioinformatics and Biostatistics, National Center for Toxicological Research, US FDA, Jefferson, AR;* [3]*Office of Scientific Coordination, National Center for Toxicological Research, US FDA, Jefferson, AR.*

Idiosyncratic drug-induced liver injury (DILI) is a major cause of drug withdrawal and can result in serious clinical outcomes, such as acute liver failure or death. The development of DILI relates not only to host idiosyncratic susceptibility factors of the treated patient, but also properties of the given drug. Current predictive models are not effective enough at identifying DILI-prone drugs or individuals, largely because of the complex and multi-layered mechanisms of the disease. Observing host factors or drug properties alone cannot give a comprehensive picture of the underlying nature of DILI. The objective of this study is to investigate the multi-layered interplay between genetic variants (GV) and drug properties related to DILI to unravel how these two components together affect the disease outcome. First, reported individual drug-genetic associations were collected through large-scale literature analysis and were grouped as immune or metabolism-related GV. Additionally, drug property data was collected through DrugBank and the FDA's Liver Toxicity Knowledge Base. Then, Fisher's exact test was used to identify robust and significant relationships between the co-occurrences of GV and drug properties and DILI risk associated with drugs. We found the co-occurrence of three combinations (immune GV + formation of reactive metabolites, metabolism GV + "Rule-of-Two", and metabolism GV + Cmax) aggravated DILI risk, associated with a higher possibility to be most DILI-concern or cause acute liver failure, when compared to GV or drug properties alone. As a proof-of-concept study, our preliminary results suggest the importance of linking host factors with drug properties to better understand DILI.

PS 3184 The Effects of Polyhexamethylene Biguanide (PHMB) on Rat Liver

A. Chowdhury[4], L. L. Arnold[4], P. R. Dodmane[4], K. L. Pennington[4], Z. Wang[3], J. E. Klaunig[3], D. Cros[1], E. E. Creppy[2] and S. M. Cohen[4]. [1]*Laboratoire Paréva, St-Martin de Crau, France;* [2]*University of Bordeaux, Bordeaux Cedex, France;* [3]*Environmental Health, Indiana University, Bloomington, IN and* [4]*Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE.*

PHMB is a non-genotoxic polymeric biocide which induced rat hepatocellular tumors in a 2 year bioassay at 1000 and 1500 ppm in water but not at 500 ppm. Studies were designed to evaluate possible modes of action. Male rats were treated with PHMB in water at 500, 1000 or 1500 ppm or in diet at 4000 ppm for 4 weeks, or 1500 ppm PHMB in water for 2 weeks. Histopathologic evaluation, staining for iron and immunohistochemical evaluation of hepatocyte and endothelial cell (EC) proliferation were done. Liver tissue and serum levels of malondialdehyde (MDA) and mitogenic cytokines and serum levels of transaminases were determined. At 2 weeks, CYP enzyme activity and expression levels of Pparα, Car and Pxr responsive genes were determined. The unpalatability of PHMB-containing water caused an early severe decrease in water and food consumption and body weight loss which reversed after 3-4 days as the rats adapted. PHMB administered in water (1000 and 1500 ppm) and in diet (4000 ppm) increased the hepatocyte and EC proliferation. There was evidence of mild centrilobular hypertrophy in rats receiving PHMB in water, but no evidence of cytotoxicity or oxidative stress-related effects in liver. No changes in serum or liver tissue cytokines, enzymes or MDA were detected. There was an increase in iron deposition in sinusoidal lining cells, most significantly in rats administered PHMB in water. PHMB had no effect on Pparα, Car and Pxr responsive gene expression or CYP enzyme activity at 2 weeks. These data support a mitogenic mode of action for PHMB-induced hepatocellular tumors, with sinusoidal iron deposition a possible contributing factor. It also suggests that the initial severe dehydration initiated the process leading to tumor development. The combination of mitogenesis, sinusoidal iron accumulation and the initial severe dehydration and weight loss indicate that the overall mode of action is not relevant to a human cancer risk.

PS 3185 Pyrrolizidine Alkaloid-induced Hepatotoxicity Involved with Binding and Functional Inhibition of ATP Synthase Beta Subunit

Y. Lu and G. Lin. *School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China.* Sponsor: N. Mei.

Pyrrolizidine alkaloid (PA) is a class of heterocyclic phytochemicals found in over 6000 plants and is a potential source of food contamination and herbal poisoning. PA is metabolized in the liver forming dehydropyrrolizidine alkaloids (DHPAs) and dehydroretronecine (DHR), which can readily react with cellular proteins forming pyrrole-protein adducts and cause liver damage. In previous studies using proteomic approach, several potential target proteins, whose level were significantly altered in the rat model of PA-induced hepatotoxicity, were identified by our group. Among them, a protein known as ATP5B, which is a critical subunit of ATP synthase that plays an important role in ATP synthesis and energy metabolism, has been further evaluated in the current study. Immunoprecipitation of ATP5B from liver tissues of PA-treated rats and mice followed by Western blot analysis with anti-pyrrole antibody revealed pyrrol-ATP5B protein adducts, which were also detectable *in vitro* using a human hepatic cell line, HepaRG. Furthermore, our results show that PA metabolites not only covalently bind to ATP5B, but also affect

the level of intracellular ATP. Following 16-hr treatment of a model PA, retrorsine, intracellular ATP level decreased by around 85% and 78% in hepatocytes and liver sinusoidal endothelial cells, respectively. Whereas cell viability of both cells had no significant change, as evaluated by the Cell Counting Kit-8 (CCK-8, Dojindo). In addition, a decrease in ATP synthase activity was also detected by ATP synthase Specific Activity Microplate Assay Kit (abcam®). Our results suggest that hepatic ATP depletion and energy crisis is closely involved in the development of the PA-induced hepatic toxicity and may be attributed to the disruption of ATP synthase function.

3186 Phenotypic Similarity of Acetaminophen-Induced Hepatotoxicity in a Monkey Model and in Human Patients

S. Tamai[1], T. Iguchi[1], N. Niino[1], K. Mikamoto[1], K. Sakurai[1], A. Sayama[1], H. Shimoda[1], W. Takasaki[1] and K. Mori[2]. *[1]Daiichi Sankyo Co., LTD., Tokyo, Japan and [2]Daiichi Sankyo Pharma Development, Daiichi Sankyo Co., LTD., Edison, NJ.*

Species-specific differences in the hepatotoxicity of acetaminophen (APAP) have been shown. To establish a monkey model of APAP-induced hepatotoxicity, which has not been previously reported, APAP at doses up to 2000 mg/kg was administered orally to fasting male and female cynomolgus monkeys (n = 3-5/group) pretreated intravenously with or without 300 mg/kg of the glutathione biosynthesis inhibitor, L-buthionine-(S,R)-sulfoximine (BSO). In all the animals, APAP at 2000 mg/kg with BSO but not without BSO induced hepatotoxicity, which was characterized histopathologically by centrilobular necrosis and vacuolation of hepatocytes. Plasma levels of APAP and its reactive metabolite N-acethyl-p-benzoquinone imine (NAPQI) increased 4 to 7 h after the APAP treatment. Additionally, BSO treatment did not affect circulating APAP and NAPQI levels. The mean Cmax level of APAP at 2000 mg/kg with BSO was approximately 200 µg/mL, which was comparable to high-risk cut-off value of the Rumack-Matthew nomogram. Interestingly, plasma alanine aminotransferase (ALT) did not change until 7 h and prominently increased 24 h or later after the APAP treatment, indicating that this phenotypic outcome was similar to that in humans. In addition, circulating liver-specific miR-122 and miR-192 levels also drastically increased 24 h or later compared with ALT, suggesting that circulating miR-122 and miR-192 may serve as more sensitive biomarkers to detect hepatotoxicity in cynomolgus monkeys. These results suggest that the hepatotoxicity induced by APAP in the monkey model shown here was translatable to humans in terms of toxicokinetics and its toxic nature, and this model would be useful to investigate mechanisms of drug-induced liver injury (DILI) and also potential translational biomarkers for diagnosis of DILI in humans.

3187 Liver Toxicity Knowledge Base (LTKB): A Comprehensive Database to Understand Multiple Dimensions of Drug-Induced Liver Injury

S. Thakkar, M. Chen, H. Fang, Z. Liu, G. Zhou, J. Zhang and W. Tong. *Division of Bioinformatics and Biostatistics, National Center for Toxicological Research, Jefferson, AR.*

Drug-Induced Liver Injury (DILI) is one of the foremost causes for acute liver failure, and a major reason for the termination of many clinical trials and discontinuation of many approved drugs. Therefore, DILI is a major concern during the process of drug development, from both the developer and the regulatory points of view. To address this issue, the Liver Toxicity Knowledge Base (LTKB) was developed, with aims of improving our understanding of the underlying mechanisms involving DILI and its prediction. This knowledgebase has collected approximately 3000 unique compounds, most of which are drugs clinically used. We also collected DILI related information such as drug physicochemical properties along with its dosage, side effect, and therapeutic uses. In addition, the database includes: mechanistically relevant cellular end points from various conventional, high-throughput and high-content *in vitro* assays, drug-elicited toxicogenomic responses from both primary hepatocytes and animals, and histopathology data. The LTKB database has links to data from the ToxCast (from EPA) and Tox21 (from NIH, EPA, NTP and FDA) projects. Drugs in the LTKB database were analyzed for its potential to cause DILI, and they were classified based on its severity. DILI annotations were also identified from the FDA- approved drug labels. These drug labels provide safety information from clinical trials and post marketing surveillance. As a result, 749 drugs were identified with some level of DILI concern and 619 drugs were further annotated for the DILI information from the NIH LiverTox database. LTKB database was developed on the Accelrys Isentris 4.0 platform. In summary, in this presentation we will exemplify, the process of extracting the desired information from database to help better understanding of DILI. This database provides comprehensive DILI information and contains the findings at various level of biological complexity at one location. In addition LTKB can be used as a resource to improve DILI predictive models for safety issues encountered in drug discovery and development.

3188 Prediction of *In Vivo* Hepatotoxicity Effects Using *In Vitro* Transcriptomics Data

J. A. Franzosa[1,2], J. Liu[1,2], R. S. Thomas[2], J. F. Wambaugh[2], K. A. Houck[2] and I. Shah[2]. *[1]ORISE, Oak Ridge, TN and [2]NCCT, US EPA, Research Triangle Park, NC.*

High-throughput *in vitro* transcriptomics data support molecular understanding of chemical-induced toxicity. Here, we evaluated the utility of such data to predict liver toxicity. First, *in vitro* gene expression data for 93 genes was generated following exposure of metabolically competent HepaRG cells to 1060 environmental chemicals from the US EPA ToxCast library. The empirical relationship between these data and rat chronic liver endpoints from animal studies in the Toxicity Reference Database (ToxRefDB) was then evaluated using machine learning techniques. Chemicals were classified as positive (242) or negative (135) based on observed hepatic histopathologic effects, and divided into three categories: hypertrophy (183), injury (112) and proliferative lesions (101). Hepatotoxicants were classified on the basis of the bioactivity of 93 genes (descriptors) using six machine learning algorithms: linear discriminant analysis, naïve Bayes, support vector classification, classification and regression trees, k-nearest neighbors, and an ensemble of classifiers. Classification performance was evaluated using 10-fold cross-validation testing, and in-loop, filter-based, feature subset selection. The best balanced accuracy for prediction of hypertrophy, injury and proliferative lesions were 0.81±0.07, 0.79±0.08 and 0.77±0.09, respectively. Gene specific perturbation of xenobiotic metabolism enzymes (CYP7A1/2E1/4A11/1A1/4A22) and transporters (ABCG2, ABCB11, SLC22A1) were most predictive of hypertrophy. The bioactivity descriptor for increased expression of NRF2, a major regulator of cell defense pathways, was highest across chemical inducers of liver injury. The results also showed that specific genes, eg. increased MYC, IL-6, and TGF-beta and decreased SULT2A1 expression, were most predictive of proliferative lesions. In all, our findings demonstrate the utility of *in vitro* transcriptomics data to predict adverse hepatic outcomes *in vivo*, and support integration of 'omics technology into hazard prioritization paradigms. This abstract does not represent US EPA policy.

3189 Epigenetic Changes in a Mouse Model of Alcohol- and Fibrosis-Associated Liver and Kidney Injury

K. Dreval[2], A. de Conti[2], S. Furuya[1], I. Rusyn[1] and I. P. Pogribny[2]. *[1]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX and [2]Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR.*

Emerging clinical evidence indicates that kidney injury is an important clinical indication of poor survival in patients with alcoholic hepatitis, an often lethal form of acute-on-chronic human disease in subjects who abuse alcohol; however, little is known about the mechanisms of alcohol-associated kidney disease syndromes. The goal of this study was to explore whether epigenetic mechanisms may play a role in the pathogenesis of fibrosis- and alcohol-associated liver and kidney damage. We used a mouse model of combined chronic treatment with carbon tetrachloride (CCl4) and intra-gastric feeding with alcohol. We found that fibrosis- and alcohol-associated liver and kidney injury in this model is associated with epigenetic changes. Specifically, liver injury was associated mainly with alterations in DNA methylation, with the greatest magnitude of DNA methylation and changes in expression of DNA methyltransferase observed in mice treated with alcohol alone or in combination with CCl4. In contrast, kidney injury was characterized by marked effects on histone marks. Particularly, levels of histone transcription-activating marks, histone H3 lysine 4 trimethylation and H3 lysine 27 acetylation, and histone DNA damage-response mark, histone H3 lysine 56 acetylation, were increased in the kidneys of mice subjected to a combined CCl4 and alcohol treatment, an experimental group that exhibited severe kidney injury. Mechanistically, these kidney-specific histone modification changes were also associated with a marked activation of genes involved in kidney injury and compromised DNA repair mechanisms. The results suggest an epigenetic component in the mechanism of alcohol-induced liver and kidney injury under conditions of associated fibrosis. Furthermore, the difference in the epigenetic hepatic and kidney responses imply tissue-specificity and severity of alcohol-induced injury.

3190 Exposure to the Genotoxic Chemical 1,3-Butadiene Confers Tissue- and Strain-specific Alterations in microRNA Expression

G. Chappell[1,2], J. Simon[2], B. Peck[2], K. Eklund[2], T. Furey[2] and I. Rusyn[1]. [1]*Texas A&M University, College Station, TX and* [2]*University of North Carolina, Chapel Hill, NC.*

MicroRNAs (miRNAs) are a class of critical posttranscriptional regulators of gene expression that can silence genes by blocking translation or inducing mRNA degradation. The role of miRNAs as mediators of toxicity has recently received increasing attention, but is not fully understood. In the present study, 1,3-butadiene (BD), a widely used industrial chemical and an environmental pollutant, was used as a model human and rodent carcinogen to study chemically-induced alterations of miRNA expression in mice. Although genotoxicity is an established mechanism of the carcinogenicity of BD, epigenetic alterations (DNA methylation and histone modifications) have also been observed in a tissue- and strain-specific manner in mice exposed to BD by inhalation. We hypothesized that tissue- and strain-specific changes in miRNA expression also occur after short-term exposure to BD, and that some miRNAs potentially regulate transcriptional response to BD. To test these hypotheses, we sequenced both mRNA and miRNA collected from the liver, lung, and kidney of male C57BL/6J and CAST/EiJ mice that were exposed to 0 or 625 ppm of BD by inhalation (6 hr/day, 5 days/week) for 2 weeks. While tissue-specific basal levels of miRNA expression were very similar between the two strains, the effect of BD on miRNA expression was more profound in C57BL/6J mice than in CAST/EiJ. This finding is in line with the previous observation that the C57BL/6J strain is more susceptible to BD-induced DNA damage and epigenetic effects. The greatest degree of change in miRNA expression was observed in the lung, followed by the kidney, and there were no differentially expressed miRNAs in the liver. Members of the miR-34/449 family, which are associated with DNA damage, were the most highly up-regulated miRNAs in the lung of BD-exposed C57BL/6J mice. Further, this family was identified as a regulatory hub based on the enrichment of mRNA targets of members of these families among the BD-induced down-regulated. Upregulation of miR-34/449 family members may explain the down-regulation of immune response genes observed in the lung of BD-exposed C57BL6 mice. Our results expand the current knowledge of miRNA responses across different tissues and strains, and present evidence for potential underlying mechanisms of BD-associated health effects.

3191 Altered Mirna Expression Profiles in Aniline-Induced Cell Cycle Regulation in Spleen

J. Wang, G. Wang and M. F. Khan. *Pathology, University of Texas Medical Branch, Galveston, TX.*

Aniline exposure is associated with toxicity to the spleen leading to splenomegaly, fibrosis, and a variety of sarcomas on chronic exposure in rats. However, early molecular events in aniline-induced cell cycle progression in the spleen remain unknown. MicroRNAs (miRNAs), short non-coding RNAs, have been implicated in tumor development through modulating key cell cycle regulators and controlling cell proliferation. This study was, therefore, undertaken on the expression of miRNAs, regulation of cyclin proteins and cyclin-dependent kinases (CDKs), in an experimental condition that precedes a tumorigenic response. Male SD rats were treated with aniline (1 mmol/kg/day, by gavage) for 7 days (controls received drinking water only), and miRNAs, cyclins and CDKs in rat spleens were analyzed. Microarray and/or qPCR analyses showed that aniline exposure led to significantly decreased miRNA expression of let-7a, miR-24, miR-34c, miR-100, miR-125b and greatly increased miR-181a in the rat spleens. The aberrant expression of miRNAs was associated with significantly increased protein expression of cyclins including cyclins A, B1, D3, E and remarkably enhanced expression of CDKs like CDK1, CDK2, CDK4, CDK6, especially p-CDK1and p-CDK2 in the splenocytes of aniline-treated rats. The data suggest that the aberrant expression of miRNAs could play an important role in the regulation of cell cycle proteins. Our findings thus, provide new insight into the role of miRNAs in cell cycle progression, which may contribute to aniline-induced tumorigenic response in the spleen. Supported by NIH ES06476.

3192 Heritable Environmental Effects Mediated by Germline Chromatin Alterations

J. A. Camacho[2], N. Gentry[2], Z. Lundby[2], L. Hosohama[2], A. Clark[2] and P. Allard[2]. [1]*Molecular Toxicology, University of California, Los Angeles, Los Angeles, CA and* [2]*Molecular, Cell and Developmental Biology, University of California, Los Angeles, Los Angeles, CA.*

The germ cell lineage is unique as it is the only cell type that bridges generations. Studying germ cell development and integrity is of great importance for health and survival, especially since these cells can be vulnerable during embryogenesis while they undergo extensive chromatin remodeling. Dynamic remodeling of chromatin imparts an epigenetic regulatory role in several biological processes. Our goal is to examine the influence of environmental chemicals on the epigenetic remodeling and chromatin silencing of germ cells. Germ cells are an especially important context to examine alterations in histone modification patterns as these cells undergo periods of profound DNA demethylation and consequently have a high reliance on histone marks to ensure genomic stability. However, these events take place in mammals early during embryogenesis which creates issues of accessibility to the cells and ease of study. Here, two compelling and innovative germ cell models are utilized: the powerful genetic system *Caenorhabditis elegans* and the *in vitro* mouse germ cell model of primordial germ cell like cells (PGCLCs). As a reporter, we are making use of a worm strain were GFP is epigenetically silenced in the germline. Our approach, the first of its kind, uses this reporter to examine the epigenetic regulation in the *C. elegans* germline, after chemical exposure. Results indicate that de-silencing effects after exposure to the environmental compounds BPA and Vinclozolin last for at least three generations (up to three-fold compared to our DMSO control). Furthermore, most worms showing de-silencing in the first, second and third generation originate from worms showing de-silencing in the parental exposed generation indicating that the effect is inherited. Our next step is to identify and characterize the toxic effects in *C. elegans* caused by chemical disruption of chromatin through environmental exposure. We also present preliminary results in PGCLCs demonstrating that this new germ cell model is applicable to chemical testing. Together, we hope to establish the role of epigenetic pathways in mediating germ cell toxicity.

3193 Cytosine DNA-Methylation Response to Aflatoxin B1 Exposure in Human Liver HepaRG Cells

V. Tryndyak[2], S. Burnett[1], F. A. Beland[2] and I. P. Pogribny[2]. [1]*University of Arkansas, Fayetteville, AR and* [2]*Division of Biochemical Toxicology, US FDA National Center for Toxicological Research, Jefferson, AR.*

Epigenetic alterations play a fundamental role in the etiology and progression of chemical-induced carcinogenesis. Global and region-specific DNA methylation changes are among the most studied epigenetic events in the carcinogenic process and have been suggested to have significance in the assessment of carcinogenicity. Thus, the development of reliable time- and cost-effective *in vitro* model systems to identify the carcinogenic potential of chemicals, with a focus on carcinogen-induced epigenetic aberrations, is of a great importance. In the present study, terminally differentiated human hepatic HepaRG cells were treated with 5 or 25 μM of genotoxic carcinogen aflatoxin B1 (AFB1) or the structurally similar compound aflatoxin B2 (AFB2) that exhibits minimal carcinogenic potential, and DNA methylation profiles were investigated using whole-genome bisulfite next-generation sequencing (WGBS) technology. Treatment of HepaRG cells with AFB1 resulted in dose-dependent cytosine DNA methylation changes at CpG sites as compared to control cells, whereas no changes in DNA methylation were found in AFB2-treated cells. Bioinformatics analysis of WGBS data from HepaRG cells treated with 25 μM AFB1 revealed 96,000 differentially methylated regions (DMRs) throughout the genome, 5201 of which were located in gene promoter regions. The methylation status of 418 of DMRs located in the gene promoters was inversely correlated with gene expression in AFB1-treated HepaRG cells, indicating a functional significance of AFB1-induced methylation changes. Ingenuity pathway analysis of these common differentially-methylated and differentially expressed genes showed a strong enrichment in genes associated with liver hyperproliferation, liver cholestasis, and hepatocellular carcinoma development. Our data demonstrate the promising value of DNA methylation alterations for identifying chemical carcinogens and understanding mechanisms of carcinogenicity.

3194 DNA Methyltransferase Expressions in Japanese Rice Fish (*Oryzias latipes*) Embryogenesis Is Developmentally Regulated and Modulated by Ethanol and 5-Azacytidine

A. K. Dasmahapatra[1,2] and I. A. Khan[1,2]. [1]*National Center for Natural Product Research, University, MS and* [2]*National Center for Natural Product Research, University of Mississippi, University, MS.*

We aimed to investigate the impact of epigenome in inducting fetal alcohol spectrum disorder (FASD) phenotypes in Japanese rice fish embryogenesis. One of the significant events in epigenome is DNA methylation which is catalyzed by DNA methyltransferase (DNMT) enzymes. We analyzed DNMT enzyme mRNA expressions in Japanese rice fish development starting from fertilized eggs to hatching and also in embryos exposed for first 48 h of development either to ethanol (300 mM) or to 5-azacytidine (5-azaC; 2mM), an inhibitor of DNMT enzyme activity. As observed in FASD phenotypes, 5-azaC exposure was able to induce microcephaly and craniofacial cartilage deformities in Japanese rice fish. Moreover, we have observed that expression of DNMTs (dnmt1, dnmt3aa, dnmt3ba, and dnmt3bb.1) are developmentally regulated; high mRNA copies were found in early stages (1-2 dpf), followed by gradual reduction until hatching. In ethanol-treated embryos, compared to controls, dnmt1 mRNA is in reduced level in 2 dpf and in enhanced level in 6dpf embryos. While dnmt3aa, 3ba, and 3bb.1 remained unaltered. In contrast, embryos exposed to 5-azaC have an enhanced level of dnmt1 and dnmt3bb.1 mRNAs both in 2 and 6 dpf embryos while dnmt3aa and 3ba are enhanced only in 6 dpf embryos. This study indicates that the craniofacial defects observed in FASD phenotypes are the results of dysregulations in DNMT expressions.

3195 Between Strain and Tissue Differences Exist in Global Hydroxymethylation After Acute Ozone Exposure

C. N. Miller, M. C. Schladweiler, J. A. Dye, M. Hazari, U. Kodavanti and I. M. Gilmour. *NHEERL, Environmental Public Health Division, US EPA, Research Triangle Park, NC.*

Epigenetics have been increasingly recognized as a mechanism linking environment and gene expression with both normal physiologic function as well as disease states. Demethylation of cysteine residues, generally leading to gene activation, is an oxygen-dependent reaction and creates the semi-stable intermediate 5-hydroxymethyl cytosine (5hmC). As air pollutants, in particular ozone, produce a strong oxidative stress response, it has been hypothesized that changes in 5hmC will occur not only in response to ozone but may also provide a novel biosensor of exposure. In the current study 12 week old, male Long-Evans and Wistar Kyoto (WKY) rats (n=32) were exposed to 6 hours of whole-body 1.0 ppm ozone or air and immediately euthanized. Global 5hmC levels were determined using an ELISA. Both WKY and Long-Evans exposed to ozone increased hydroxymethylation within the liver compared to air controls ($p<0.05$). In adipose tissue, an acute ozone exposure resulted in global reductions in 5hmC within both strains compared to their air controls ($p<0.06$). A strain difference became apparent in the response within the lung. Long-Evans rats increased global 5hmC levels in the lung compared to controls, with no difference measured in the WKY rats. Previous work has shown that relative to many other strains, ozone increases bronchoconstriction and vitamin C sequestration in the WKY strain. These findings suggest that total ozone dose and oxidative damage may be reduced in WKY rats. Our study provides the first causal evidence that an inhaled pollutant induces systemic changes to 5hmC levels in both a tissue and strain-dependent manner. Further, we suggest strain differences may be due to differing antioxidant defense systems within the lung. Lastly, we recommend that researchers adopt a broad approach to tissue screens when considering 5hmC as a biomarker of exposure due to the inherent differences we measured. This abstract does not reflect US EPA policy.

3196 Effects of Developmental Lead Exposure on the Hippocampal Methylome: Influences of Sex and Timing and Level of Exposure

J. Schneider[2], G. Voisin[1], F. Lefebvre[1] and D. Anderson[2]. [1]*Atelerics, Montreal, QC, Canada and* [2]*Thomas Jefferson University, Philadelphia, PA.*

Developmental lead (Pb) exposure to results in persistent cognitive/behavioral impairments as well as an elevated risk for developing a variety of diseases in later life. Developmental environmental exposures can result in a variety of epigenetic changes, including alterations in DNA methylation that can influence gene expression patterns and affect the function and development of the nervous system. The present methylation microarray profiling study explored the extent to which developmental Pb exposure may modify the hippocampal methylome. Furthermore, interactive influences of sex, level of Pb exposure and developmental period of Pb exposure were studied. The hippocampus was chosen for study given its sensitivity to the effects of Pb exposure in rodents and humans. 126 Long Evans rats, 63 male and 63 female, were used. Exposure levels were 0 ppm, 150 ppm, 375 ppm, or 750 ppm, provided as Pb acetate in food. Animals had perinatal Pb exposure (gestation through lactation), early postnatal exposure (birth through weaning), or extended postnatal exposure (birth through postnatal day 45). All animals were euthanized at day 55, hippocampi were rapidly removed and DNA processed for hybridization to Rat NimbleGen 3x720K CpG Island Plus RefSeq Promotor Arrays. Data were normalized and probe-wise differential methylation analysis between experimental groups was performed, followed by gene ontology (GO) and pathway analyses. Redundancy analysis, an ordination approach, was also performed to interpret differentially methylated CpG sites across the multifactorial experimental design. Results show an overwhelming significant contribution of sex to the hippocampal methylome with complex interactions of timing of exposure and level of exposure, with at least 80% of the variance explained by sex and Pb exposure level. Surprisingly, the developmental period of exposure contributed only a small amount of variance to the overall data and GO analysis revealed the largest number of overrepresented GO terms in the lowest exposure group. These data reinforce the significant effect that low level Pb exposure has on brain DNA methylation patterns. Supported by NIH grant ES015295

3197 Free Radical-Mediated Cytosine C-5 Methylation by Environmental Chemicals

K. Kawai, H. Kasai, Y.-S. Li and M.-F. Song. *Department of Environmental Oncology, University of Occupational and Environmental Health, Kitakyushu, Japan.*

Epigenetic disorders have recently been drawing keen attention as a mechanism of carcinogenesis. For example, hypermethylation of a tumor suppressor gene can lead to gene silencing. Although DNA methyltransferases are involved in the hypermethylation, many points still remain unclear about how tumor suppressor gene promoters become hypermethylated in the carcinogenic process. We have been studying the methylation of the C-5 position of deoxycytidine, in order to clarify the relationship between environmental chemicals and cytosine C-5 methylation. We found that the C-5 position of cytosine can methylated by cumene hydroperoxide or tert-butylhydroperoxide in the presence of Fe(II). These reactions proceed via methyl radical formation. Both compounds are widely used in industrial applications and are known tumor promoters. We also demonstrated the methylation of cytosine by dimethylsulfoxide and methionine sulfoxide, in the presence of the Fenton reagent. Our studies suggested that free radical-mediated cytosine C-5 methylation is a possible mechanism of epigenetic disease. Cytosine C-5 methylation should be focused on to determine the roles of environmental chemicals in epigenetic alterations, because inflammation, smoking, aging, nickel and arsenic have been reported as hypermethylation factors in carcinogenesis.

3198 Chronic Exposure to Trichloroethylene Increases Plasticity of *Ifng* Expression and DNA Methylation Variance in CD4+ T Cells

K. Gilbert[3], S. Blossom[3], S. Erickson[3], B. Reisfeld[2], T. Zurlinden[2], B. Broadfoot[3], K. West[3], S. Bai[3] and C. Cooney[1]. [1]*Central Arkansas Veterans Healthcare System, Little Rock, AR;* [2]*Colorado State University, Fort Collins, CO and* [3]*University of Arkansas for Medical Sciences, Little Rock, AR;* [4]*Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR;* [5]*Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR.*

Exposure to solvent and water pollutant trichloroethylene (TCE) has been linked to increased incidence of autoimmunity in humans. Using a mouse model we have shown that chronic TCE exposure induces autoimmune hepatitis (AIH), and among immune system components, appears to primarily impact CD4+ T cells. Human autoimmune diseases represent long-term processes that encompass relapsing-remitting pathology and multi-phasic immune system alterations. The current study focused on time-dependent changes in CD4+ T cells as a way of defining the etiology of TCE-induced AIH. Both effector/memory and naïve CD4+ T cells were isolated from female MRL+/+ mice every 6 weeks during a 40 week exposure to TCE (0.5 mg/ml in drinking water). Transcriptomic analysis of effector/memory CD4+ T cells showed that 22 weeks of TCE exposure altered over 400 genes with the increased gene expression

centered on Ifng. However, 40-week exposure to TCE decreased expression of Ifng and several other cytokine genes upregulated at the earlier time point. Bisulfite DNA sequencing showed that over time TCE increased DNA methylation of CpG sites in the promoter region of Ifng in effector/memory CD4+ T cells. Aside from overt hyper- or hypo-methylation more subtle epigenetic effects include alterations in the variance in the DNA methylation of specific CpG sites. When data from individual genes was fit to a quadratic regression model TCE was shown to alter gene-specific methylation variance in both naïve and effector/memory CD4+ T cells. This longitudinal study suggested that a TCE-induced increase in methylation plasticity may help regulate the non-monotonic effects of TCE on CD4+ T cells during disease development.

3199 Epigenetics and Preeclampsia: Defining Functional Epimutations in the Preeclamptic Placenta Related to the TGF-β Pathway

E. Martin[2], P. D. Ray[2], L. Smeester[2], M. R. Grace[2], K. Boggess[2] and R. C. Fry[2]. [1]*Curriculum in Toxicology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC and* [2]*Department of Obstetrics & Gynecology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC;* [3]*Environmental Science and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Preeclampsia is a potentially fatal pregnancy disorder affecting millions of women around the globe. Dysregulation in gene and protein expression within key biological pathways controlling angiogenesis has been implicated in the development of preeclampsia. Altered CpG methylation, a type of epimutation, may underlie this pathway dysregulation. In the present study, placental tissue from preeclamptic cases and normotensive controls was analyzed for genome-wide differential CpG methylation and concomitant changes in gene expression. A set of 123 genes, representing 19.9% of all genes with altered CpG methylation, was associated with functional changes in transcript levels. Underscoring the complex relationships between CpG methylation and gene expression, here hypermethylation was never associated with gene silencing, nor was hypomethylation always associated with gene activation. Moreover, the genomic region of the CpG mark was important in predicting the relationship between CpG methylation and gene expression. The 123 genes were enriched for their involvement in the transforming growth factor beta (TGF-β) signaling pathway, a known regulator of placental trophoblast invasion and migration. This is the first study to identify CpG hypomethylation as an activator of TGF-β-associated gene expression in the preeclamptic placenta. The results suggest functional epimutations are associated with preeclampsia disease status and the identified genes may represent novel biomarkers of disease.

3200 DNA Methylation and Transcriptional Changes in Smokers During Monocyte Differentiation

N. A. Englert, M. Campbell, C. Crowl, G. Pittman, D. Porter and D. A. Bell. *Genome Integrity and Structural Biology Laboratory, NIEHS, Research Triangle Park, NC.*

The cellular response to environmental exposures such as cigarette smoke involves activation of the xenobiotic metabolism pathway and immune response. Cigarette smoke exposure also reduces DNA methylation in the aryl hydrocarbon receptor repressor (AHRR) gene at cg05575921 and at nearby CpGs, and is associated with increased AHRR expression in human population studies. To better understand how DNA methylation and gene expression levels of AHRR in smokers (SM) and nonsmokers (NS) are affected during hematopoiesis, we developed an *ex vivo* model of monocyte differentiation into terminal dendritic cells or macrophages. We hypothesized that smoking-induced methylation changes in AHRR would be maintained through differentiation. Circulating CD14+ monocytes were isolated from 4 NS and 6 SM volunteers and cultured for 5 days in the presence of GM-CSF and autologous serum. Resulting monocytes, macrophages, and dendritic cells were sorted by FACS, and DNA methylation was analyzed by high resolution melt PCR. NS tended to have more dendritic cell formation as well as higher AHRR methylation in monocytes compared with SM (average 43%). Interestingly, macrophages exhibited a similar methylation difference between NS and SM, indicating methylation differences remain consistent through differentiation processes. AHRR expression levels in monocytes and macrophages were on average higher in SM than NS. Our data support the hypothesis that smoking-induced methylation differences at AHRR cg05575921 are maintained through differentiation and follow-up studies are in progress. Other cell types, including hematopoietic progenitor cells, should be assayed to further test the hypothesis. Continued studies of FACS cell populations (NS vs. SM) include genome-wide differential methylation analysis using reduced representation bisulfite sequencing (RRBS) and transcriptomic analysis using whole transcriptome microarrays.

3201 Formaldehyde Facilitates Cell Transformation by Compromising Chromatin Assembly

D. Chen[3], H. Li[1], L. Fang[3], J. Liu[3], K. Lu[2] and C. Jin[3]. [1]*Department of Pathology, SUNY Downstate Medical Center, New York, NY;* [2]*Environmental Health Science, University of Georgia, Athens, GA and* [3]*Environmental Medicine, NYU School of Medicine, Tuxedo, NY.*

Formaldehyde (FA) is an environmental and occupational chemical carcinogen. The molecular mechanisms of FA-induced carcinogenesis are not fully understood, with little known about the molecular mechanisms responsible for FA-induced epigenetic dysregulation. Histone modifications such as lysine acetylation, are crucial for both chromatin function and the assembly/disassembly of chromatin. Defective chromatin assembly has been directly linked to different types of cancers. Nonetheless, the effect of chemical carcinogens on chromatin assembly has not been studied. It has been known that FA reacts with lysine residues on histone proteins to form a labile Schiff base or the more stable N6-formyllysine and that both Schiff bases and N6 formyllysine residues are refractory to acetylation. The later finding indicated that lysine formylation might interfere with physiologically important signaling processes regulated by lysine acetylation. To investigate what kind of chromatin function(s) are affected by FA adducts to histone lysine residues, we first detected subcellular changes of histone modifications by cellular fractionation and Western blot analyses. We found a drastic decrease of acetylation of N-terminal tails of the cytosolic histones H3 and H4 following FA exposure. The finding is intriguing, given that acetylation of histones is important for their nuclear import and assembly into chromatin. In fact, the cellular fractionation analyses revealed that the amount of histone proteins in chromatin fraction decreased following FA exposure. The chromatin immunoprecipitation (ChIP) assays further showed histone depletions at most of the genomic loci we tested, suggesting that FA compromises chromatin assembly. Notably, FA exposure led to changes in chromatin accessibility and the expression of several oncogenes and tumor suppressor genes as determined by micrococcal nuclease (MNase) digestion assay and RNA-seq. Importantly, the knockdown of a histone gene, which mimics partial inhibition of chromatin assembly, induced and facilitated FA-mediated anchorage-independent cell growth. We propose that reacting with newly synthesized histones thereby inhibiting histone nuclear import and chromatin assembly may represent a novel mechanism for chemical carcinogenesis.

3202 Mechanistic Studies on As3+ -Mediated Alteration of Histone Acetyltransferase TIP60

L. M. Tam, Q. Cai and Y. Wong. *Department of Chemistry, University of California Riverside, Riverside, CA.*

Arsenic is a prevalent environmental pollutant, generated both naturally and anthropogenically from certain industrial processes. Arsenic pollution is widespread in groundwater source especially in the Mid-West region of the United States and India. Epidemiological studies have revealed that arsenic exposure to drinking water is significantly associated with the prevalence of skin, bladder and lung cancers. Previous studies showed that arsenite could compromise DNA repair via targeting the cysteine residues in the C3H and C4 types of zinc fingers of DNA repair proteins and histone E3 ubiquitin ligases, which inhibits DNA repair directly or indirectly via inducing a chromatin state unconducive to DNA repair. We hypothesize that As(III) may also bind to the sulfhydryl group of cysteine residues in C3H-type of zinc finger motifs of histone acetyltransferase (HAT) TIP60, thereby perturbing epigenetic pathways. Here, we report that 24-hour treatment with sodium arsenite in different doses (0, 1, 2 or 5 μM) results in the dose-dependent decrease of the protein level of TIP60 HAT in HEK293T cells. This is in line with the preliminary data showing that treatment with 5 μM sodium arsenite led to a significant decline in TIP60 HAT activity, as indicated by a HAT assay. This result showed that arsenite causes the decrease in amount of TIP60 protein which in turn diminishes the overall acetylation activity (H4K16Ac). H4K16 acetylation mark (H4K16Ac) is known to open chromatin structure for ATM activation and HR repair. We also found that co-treatment with 4 μM MG-132, a 20S proteasome inhibitor, could significantly inhibit the As(III)-induced dose-dependent decrease in the amount of TIP60 protein, suggesting that the ubiquitin proteasome pathway might be involved in regulating the amount of TIP60 upon arsenite exposure. We are now in the process of further exploiting the molecular mechanisms underlying the arsenite-induced decrease in TIP60 protein. In par-

ticular, streptavidin agarose affinity pull-down assay with the use of a biotin-As(III) probe is under way to reveal whether As(III) interacts directly with TIP60 in HEK293T cells. Taken together, we conclude that arsenite interacts with cysteine residues of RING finger motifs of HAT TIP60 which enhances its degradation toward ubiquitin-proteasome pathway, decreasing histone acetylation marks and hence inhibiting DSB repair. This provides a potential mechanism for arsenic-induced carcinogenesis.

3203 Longitudinal Effects of Perinatal Bisphenol A Exposure and Variable Diet on Epigenetic Drift in Mice

J. Kochmanski[2], M. Savidge[2], L. Marchlewicz[2], C. Faulk[1] and D. C. Dolinoy[2]. [1]*Animal Science, University of Minnesota, St. Paul, MN and* [2]*Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

The epigenome is a dynamic regulatory framework that utilizes epigenetic information (e.g. DNA methylation) to govern the response of cells, tissues, and individuals to environmental stressors. DNA methylation, while transferrable mitotically, is also dynamic, and demonstrates age-dependent changes, or epigenetic "drift." Environmental factors, including chemicals and diet, have been shown to affect DNA methylation across the epigenome, but the effects of exposures on drift remain unclear. Here, we utilized a longitudinal mouse exposure study to test the hypothesis that early life exposure to the endocrine-disrupting chemical, bisphenol A (BPA), and/or variable diet, results in altered epigenetic drift, as measured by longitudinal DNA methylation at target genes in paired mouse tail tissue (3 wks/10 mos old). DNA methylation was quantified at a global repetitive element (LINE-1) and two imprinted genes (Igf2, H19) in isogenic mice perinatally exposed to Control, BPA (50 µg/kg), Mediterranean, Western, Mediterranean+BPA (50 µg/kg), or Western+BPA (50 µg/kg) diets. Absolute tail DNA methylation levels significantly increased between 3 wks and 10 mos at Igf2 (p=3.90E-5), but significantly decreased at H19 (p=4.09E-24) and LINE-1 (p=3.22E-11). Dietary exposure group did not modify drift direction in LINE-1 and H19, but Igf2 demonstrated diet-specific modification of drift. At the imprinted Igf2 locus, there were significant or near-significant decreases in DNA methylation over time in the Control (p=0.007), BPA (p=0.006), Mediterranean (p=0.08), and Mediterannean+BPA (p=0.001) groups, but this pattern was absent in the Western (p=0.69) or Western+BPA (p=0.94) groups, indicating that exposure to a Western diet affects epigenetic drift across the life-course. This study demonstrates measurable, gene-specific epigenetic drift, as well as diet-dependent alterations in the rate of drift at an imprinted locus related to murine growth and development, a phenomenon that should also be evaluated in human cohorts.

3204 Integrative Analysis of Epigenetics and Metabolomics Using the ELEMENT Cohort

J. M. Goodrich[3], E. Hector[3], L. Tang[3], P. X. Song[3], D. C. Dolinoy[3], E. H. Marchlewicz[3], A. Mercado-Garcia[6], B. Sanchez[3], H. Hu[2], M. M. Tellez-Rojo[6] and K. E. Peterson[3]. [1]*Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI;* [2]*Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada;* [3]*Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI;* [4]*Environmental Health Sciences and Nutritional Sciences, University of Michigan School of Public Health, Ann Arbor, MI;* [5]*Nutritional Sciences, University of Michigan School of Public Health, Ann Arbor, MI and* [6]*Statistics, Center for Evaluation Research and Surveys, National Institute of Public Health, Cuernavaca, Mexico.*

Epigenetic changes accumulated from early life exposures may contribute to physical growth and metabolic phenotypes in adolescents. In an association study, we analyzed the correlation of DNA methylation with plasma metabolite levels in peripubertal children (ages 8-14 years) based on a cross-sectional set of data and samples (n=246) from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) cohort. ELEMENT is a birth cohort study of mother-child pairs recruited during pregnancy and followed through adolescence and beyond. One of the central themes of ELEMENT is to assess whether the impact of prenatal lead (Pb) exposure on growth and risk for metabolic syndrome in adolescents and adults is related to epigenetic change. Three metabolites (C9.H2.N.P.S2, X5.oxoproline, and xylose) from untargeted metabolomics analysis were selected as being robustly associated with maternal patella Pb, a measure of cumulative *in utero* exposure (at q<0.05 adjusted for multiple comparisons). These metabolites were then analyzed for association with percent DNA methylation in adolescent blood leukocytes at LINE-1 repetitive elements and three growth-related genes (IGF2, H19, HSD11B2). Sex- and CpG site-specific associations were observed between metabolites and DNA methylation after adjusting for age, gender, and maternal bone Pb. For example, increased methylation in the differentially methylated region of imprinted gene IGF2 (at CpG sites 1 and 2) had a suggestive association with increased xylose among boys (p=0.09). Methylation at CpG site 3 in LINE-1 was inversely associated with xylose among girls (p=0.04). Long term, this research aims to identify pathways through which early life exposures to toxicants such as Pb influence risk for metabolic syndrome as children develop into adults.

3205 Assessment of Global and Gene-Specific DNA Methylation in Rat Liver and Kidney in Response to Non-Genotoxic Carcinogen Exposure

S. Özden[5], N. Turgut Kara[4], O. U. Sezerman[3], I. M. Durasi[2], T. Chen[6], J. K. Chipman[1] and A. Mally[7]. [1]*School of Biosciences, University of Birmingham, Birmingham, United Kingdom;* [2]*Department of Biological Sciences and Bioengineering, Faculty of Engineering and Natural Sciences, Sabancı University, Istanbul, Turkey;* [3]*Department of Biostatistics and Medical Informatics, Acibadem University, Istanbul, Turkey;* [4]*Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey;* [5]*Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey;* [6]*Department of Toxicology, School of Public Health, Soochow University, Suzhou, China and* [7]*Department of Toxicology, University of Wuerzburg, Wuerzburg, Germany.*

Altered expression of tumor suppressor genes and oncogenes, which is regulated in part at the level of DNA methylation, is an important event involved in non-genotoxic carcinogenesis. This may serve as a marker for early detection of non-genotoxic carcinogens. Therefore, we evaluated the effects of non-genotoxic hepatocarcinogens, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), hexachlorobenzene (HCB), methapyrilene (MPY) and male rat kidney carcinogens, d-limonene, p-dichlorobenzene (DCB), chloroform and ochratoxin A (OTA) on global and CpG island promoter methylation in their respective target tissues in rats. No significant dose-related effects on global DNA hypomethylation were observed in tissues of rats compared to vehicle controls using LC-MS/MS in response to short-term non-genotoxic carcinogen exposure. Initial experiments investigating gene-specific methylation using methylation-spesific PCR and bisulfite sequencing, revealed partial methylation of p16 in the liver of rats treated with HCB and TCDD. However, no treatment related effects on the methylation status of Cx32, e-cadherin, VHL, c-myc, Igfbp2, and p15 were observed. We therefore applied genome-wide DNA methylation analysis using methylated DNA immunoprecipitation combined with microarrays to identify alterations in gene-specific methylation. Under the conditions of our study, some genes were differentially methylated in response to MPY and TCDD, whereas d-limonene, DCB and chlorofom did not induce any methylation changes. 90-day OTA treatment revealed enrichment of several categories of genes important in protein kinase activity and mTOR cell signaling process which are related to OTA nephrocarcinogenicity.

3206 Differential Effects of DNA Methylation in Malignant Transformation of BEAS-2B Cells Induced by Radon and Cigarette Smoking

J. Li[2], W. Fan[1] and Y. Ji[2]. [1]*Hope Industry & Trade Co., Ltd, Tianjin, China and* [2]*Toxicology, Soochow University, Suzhou, China.*

To investigate the mechanisms of lung cancer caused by radon and cigarette smoking. Method: The immortalized human bronchial epithelial cells (BEAS-2B) in exponential growth phase were planted onto Transwell membrane. Cells were directly exposed to radon and cigarette smoking. Biological characteristics between the control group and different passages cells were compared for cell cycle and apoptosis, the extend of malignant transformation was detected by soft agar colony formation. The mRNA expression of DNMT1, DNMT3A, DNMT3B were detected by Q-PCR. Using Illumina450k methylation chip screened abnormal methylation specific genes. Results 1)The survival rates of BEAS-2B cells were related to concentration and time of exposure. Appropriate exposure of radon was 20000Bq/m3 and 30mins and of cigarette smoking was 20% and 10min. A series of sequential steps emerged among cells which exposed to radon and cigarette smoking for 15 to 20 passages, showed similar characteristics of malignant cells. The percentage of G1 phase percentage in the radon group cells was significantly lower and the percentage of S phase was significantly higher. 2) The apoptosis were significantly lower in cells after generation. The capacity of colony formation rate was significantly lower in combined group compared to control group and was up to 24.00%±0.31% in soft ager after exposure for 15 passages and generation for 20 passages(P<0.05). 3) The Genome-wide methylation level were higher in the control

group than the exposure group. The mRNA level of DNMT1 gene was higher in the control group than the exposure group, The DNMT3A and DNMT3B gene expression was lower in the control group than the exposure group. 4)Using Illumina450k methylation chip screened abnormal methylation specific genes. CDK5RAP1 genes were much lower in the combined group, SPDEF genes were highly expressed in the single group, ABCG1 genes were highly expressed in the radon group, PTPRM genes were much lower in the cigarette smoking group. Conclution 1) A model of malignant transformation of human cells *in vitro* induced by radon and cigarette smoking was established 2) Methylation status may have changed due to DNMTs genes expression alteration during the malignant transformation. 3) Abnormal methylation genes may play different role during the malignant transformation of BEAS-2B cells induced by radon and cigarette smoking *in vitro*.

3207 Screening for miRNA Regulation of Cytochrome P450 4V2, the Gene Associated with Bietti's Crystalline Dystrophy

C. M. Lockhart and E. J. Kelly. *Pharmaceutics, University of Washington, Seattle, WA.*

Bietti's Crystalline Dystrophy (BCD) is a rare, degenerative eye disease caused by mutations in the cytochrome P450 4V2 (CYP4V2) gene. The disease is characterized by yellow-white crystalline deposits on the cornea and retina, and patients will eventually become completely blind. Beyond BCD, we are interested in the function of CYP4V2 and the potential for interindividual variation in normal physiology. Given that the CYP4V2 transcript has a particularly long 3' UTR (2.8 kb), we are exploring the potential for epigenetic regulation by micro RNA (miRNA). Methods: Microarray analysis of the UW School of Pharmacy Human Liver Bank was conducted to identify miRNA candidates with potential to regulate CYP4V2. Tissues with known high and low expression of CYP4V2 mRNA were screened to identify potential miRNAs and determine regulatory associations. To evaluate the mechanism of miRNA regulation *in vitro*, HepG2 cells were stably transfected with a luciferase reporter vector containing the entire human CYP4V2 mRNA 3'UTR or a negative control vector. Multiple clones from each construct have been dually transfected with clones to either over-express or inhibit miR-146b. Luciferase was measured and compared to baseline measurements. Results: A miRNA microarray analysis identified a negative association between miR-146b-5p and CYP4V2 mRNA. Consistent with the expected translational suppression of CYP4V2 mRNA by miRNA, Western Blot analysis verified CYP4V2 protein expression was positively correlated with CYP4V2 mRNA ($R2 = 0.472$; $p = 0.0282$), and negatively correlated with miR-146b-5p ($R2 = 0.4254$; $p = 0.041$). There was no significant difference in luciferase activity between clones containing the CYP4V2 3'UTR and the scrambled control at baseline. Measurements are ongoing with clones transfected to over-express or inhibit miRNA. Discussion: We demonstrated an association between miR-146b-5p, CYP4V2 mRNA levels, and protein expression. The availability of reporter cell lines will allow testing of the function of miR-146b-5p via targeted over-expression or repression of this miRNA. To validate results from transfected HepG2 cells, mRNA and protein expression will be analyzed in primary human hepatocytes under the same experimental conditions. miR-146b is associated with inflammatory processes, bringing up the possibility that CYP4V2 may play a regulatory role in these signaling pathways in normal physiology and ocular toxicology.

3208 Epigenetic Alterations in Human Liver HepaRG Cells Induced by Aflatoxin B1 Exposure

S. Burnett[1], V. Tryndyak[2], F. A. Beland[2] and I. P. Pogribny[2]. [1]*University of Arkansas, Fayetteville, AR and* [2]*Division of Biochemical Toxicology, US FDA National Center for Toxicological Research, Jefferson, AR.*

Epigenetic alterations induced by exposure to carcinogenic agents are fundamental early events in the carcinogenic process and play an important role in cancer etiology and progression. In our previous study, we demonstrated that exposure of terminally differentiated human liver HepaRG cells to the genotoxic carcinogen aflatoxin B1 (AFB1) resulted in significant changes in the CpG island DNA methylation pattern characterized by both hypermethylation and hypomethylation. In the present study, we investigated potential underlying mechanisms associated with AFB1-induced changes in DNA methylation. Treatment of HepaRG cells with AFB1 resulted in an increased expression of DNA methyltransferase genes, DNMT1, DNMT3A, and DNMT3B, and elevation of DNMT3A and DNMT3B proteins. In contrast the level of DNMT1 protein was markedly reduced in AFB1-treated cells. In addition to DNA methylation changes, treatment of HepaRG cells with AFB1 resulted in prominent changes in histone modifications, including a loss of the trimethylation of histone H3K9, and H4K20, and hypoacetylation of histone H3K9, H3K27, and H4. These histone modification changes were accompanied by a reduction of chromatin-modifying proteins, including histone deacetylase HDAC1. In summary, the results of the present study demonstrate clearly the existence and importance of early non-genotoxic epigenetic events, in addition to a well-established genotoxic changes, in the mechanism of AFB1 carcinogenicity. Mechanistically, AFB1-induced epigenetic changes may be attributed and related to a heavily compromised functioning of chromatin modifying machinery.

3209 The DNA Methylome of Chronic Doxorubicin Exposure *In Vivo*

K. K. Nordgren[1], M. Hampton[2] and K. B. Wallace[1]. [1]*Biomedical Science, University of Minnesota Medical School Duluth campus, Duluth, MN and* [2]*Mathematics and Statistics, University of Minnesota Duluth, Duluth, MN.*

Doxorubicin (DOX) is a widely used treatment for a broad scope of human cancers but carries an increased risk of life-threatening congestive heart failure (CHF). DOX-induced mitochondrial damage is cumulative and persistent, similar to that observed clinically for the risk of CHF. Recent evidence suggests the persistent nature of this injury is caused by altered regulation of genes important to normal cardiac functioning. We hypothesize that persistent mitochondrial toxicity (and thus CHF risk) induced by DOX therapy is the result of epigenetic alterations to the DNA methylation (meDNA) status of critical regulators of mitochondrial function and capacity. Previously we showed that Sprague Dawley rats receiving six weekly injections of DOX (2 mg/kg, s.c.) show increased rates of reactive oxygen species formation and DNA oxidation in cardiac myocytes and a strong inhibition of mitochondrial respiration and calcium loading capacity, all of which persisted for at least 5 weeks following the last DOX injection and are indicators of a persistent compromise of mitochondrial function. Using this same model, we performed Reduced Representation Bisulfite Sequencing on cardiac tissues in order to map specific sites of genomic DNA methylation in both treated and control animals. Comparison of these methylomes, using the bisulfite mapper Bismark, indicated that DOX exposure alters the DNA methylation landscape, and identified 14 novel genes with highly altered methylation status. Preliminary functional effects of DNA methylation changes were characterized by quantifying mRNA expression via RT-qPCR of five selected targets (Rbm20, Nmnat2, Klhl29, Cacna1c, Scn5a). Gene expression of Rbm20 and Nmnat2 were significantly altered in the DOX treated animals (197% and 31% of control, respectively), which is consistent with the large changes in DNA methylation of these same genes. In conclusion, through an epigenotype-to-phenotype approach, this study identifies potential epigenetic markers and molecular regulators of the irreversible DOX-induced mitochondrial toxicity associated with the clinically limiting CHF. (Supported in-part by grants from the Whiteside Institute and MN Drive).

3210 Stable Transcriptional Alterations by Nickel Through Epigenetic Reprogramming

S. Cuddapah, C. Jose and L. Jagannathan. *Environmental Medicine, New York University School of Medicine, Tuxedo, NY.*

Nickel compounds are environmental carcinogens associated with the development of lung and nasal cancers in humans. Although Ni is a proven carcinogen, its mutagenic potential is low and the molecular basis of Ni-induced carcinogenicity is not fully understood. Growing evidence suggests alterations to epigenetic landscape due to Ni exposure as a risk factor for disease development. In this study, using RNA-Seq and chromatin immunoprecipitation (ChIP) analysis, we comprehensively analyzed Ni-induced alterations to transcriptional program and chromatin modifications in the immortalized non-cancerous human lung epithelial, BEAS-2B cells. Our results show that Ni exposure resulted in differential expression of several genes (≥2 fold). The expression of several Ni-affected genes returned to normal levels when the Ni-exposed cells were cultured without Ni for several generations (Ni-washed out cells). However, a subset of genes remained differentially expressed even in the Ni-washed out cells, indicating persistent changes to the transcriptional program. Interestingly, chronic Ni-exposure resulted in more genes being persistently differentially expressed, compared to acute exposure. To understand the significance of long-term gene expression alterations, we next investigated the disease association of the persistently differentially expressed genes. Our analysis showed that the persistently differentially expressed genes were associated with several diseases including cancer, inflammatory, dermatological and respiratory diseases. These results suggest the importance of Ni-induced permanent gene expression alterations in disease processes. Investigation of the epigenetic features of the differentially expressed

genes in Ni-exposed and Ni-washed out cells revealed stable alterations in histone modification profiles, suggesting epigenetic reprogramming as a basis for long-term gene expression alterations induced by Ni exposure. Supported by NIH, NIEHS grants R01ES023174, R01ES024727 and P30ES000260.

3211 Environmental Contaminants and microRNA Regulation: Transcription Factors as Master Regulators of Toxicant-Altered microRNA Expression

J. J. Sollome[2], E. Martin[2], P. Sethupathy[2] and R. C. Fry[2]. [1]*Curriculum in Toxicology, School of Medicine, University of North Carolina, Chapel Hill, NC and* [2]*Department of Genetics, School of Medicine, University of North Carolina, Chapel Hill, NC;* [3]*Environmental Health Sciences and Engineering, University of North Carolina, Chapel Hill, NC.*

MicroRNAs (miRNAs) regulate gene expression in normal human physiological processes and in pathological conditions. They bind to mRNA transcripts and regulate gene expression by inhibiting translation and/or inducing degradation of the associated transcripts. Expression levels of miRNAs have been shown to be altered by environmental toxicants, impacting normal physiology and influencing disease risk. Transcription factors (TFs) play a critical role in the regulation of miRNAs, but it is not known which TFs are most relevant for controlling miRNAs in response to different environmental toxicants. *In silico* analysis of genomic sequences adjacent to transcription start sites (TSS) of miRNAs is an efficient approach for identifying candidate TFs that regulate miRNA expression. This methodology significantly narrows the field of potential TFs that could be investigated experimentally. In this study, we set out to identify TF binding sites (TFBS) that are over-represented within promoter regions of miRNAs that are dysregulated by various environmental toxicants. A total of 13 environmental contaminants/stressors have been reported to influence the expression of miRNAs in a variety of tissues and biological samples. Based on the data from these studies, we generated a database of environmentally-modulated miRNAs (n=128) that links each contaminant with miRNAs and the tissue(s) of effect. We then analyzed the promoter sequences of these 128 miRNAs and identified a significant over-representation of binding sites for 75 TFs across ten different contaminants from one or more studies. While most TFs differed across environmental contaminants, one TF was common to nine environmental contaminants, namely SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 3 (SMARCA3). These identified TFs represent candidate master regulators of miRNAs perturbed by environmental toxicants and merit further experimental validation.

3212 Epigenetic Regulation of the MicroRNA, mir-21, in Mice Exposed *In Utero* to Second-Hand Smoke and Challenged as Adults with Ovalbumin

H. M. Zaman[1], Z. Perveen[1], R. Xiao[2] and A. Penn[1]. [1]*Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, LA and* [2]*Department of Anesthesiology, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY.*

In utero second-hand smoke (SHS) exposure activates pro-asthmatic and oncogenic microRNAs (miRNAs), including miR-18a, miR-21, miR-155, in ovalbumin (OVA)-challenged adult male & female mice (Xiao et al, 2015, submitted). Overexpression of these oncogenic miRNAs was associated with downregulation of tumor suppressor genes and thus may contribute to lung cancer progression. We investigated epigenetic regulation of miR-21 in our test system by studying the methylation status at CpG islands (CGI) upstream of the transcription start sites (TSS). We diluted SHS from 3R4F filtered research cigarettes (University of Kentucky) with HEPA-filtered air (final particle conc. = 10 mg/m3). We exposed pregnant Balb/c mice to SHS, GD 6-19. All offspring were sensitized by OVA injections at 19 & 21 weeks, followed by OVA aerosol challenge every other day in weeks 22 & 23, before sacrifice. After bisulfite treatment of lung DNA extracts, the CGI-2 sequence, located 2 kb upstream of TSS, was analyzed for methylation status with the help of the BIQ Analyzer. *In utero* SHS treatment resulted in reduction of methylated CpG cytosines in female mice at all of the eight CpG sites present in CGI-2. The average demethylation at these CpG sites was 40% while at individual CpG sites it ranged from a low of 13% to a high of 63%. In male mice, a small increase in methylation was observed (average of 13%). We conclude that miR-21 is epigenetically regulated in our test system. Since miR-21 regulates expression of 7 tumor suppressor genes that were all down-regulated in lungs of adult SHS-OVA mice compared with Air-OVA mice, these results suggest that SHS exposure *in utero* may promote a pro-oncogenic environment in lungs of adult mice, at least in part due to altered methylation status of miR-21.

3213 Diethylstilbestrol (DES) Induces Transgenerational Dysregulation in Thymic Microrna with Significant and Long-Term Consequences on Immune Functions

N. P. Singh, U. P. Singh, M. Nagarkatti and P. Nagarkatti. *Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC.*

Diethylstilbestrol (DES) is an endocrine disruptor and has been shown to cause transgenerational toxicity in humans. Prenatal exposure to DES causes thymic atrophy, affects thymic T cell differentiation, and causes changes in immune functions and thereby potentially increase susceptibility to cancer and autoimmune diseases. In the current study, we investigated the effect of DES on microRNA (miR) profile in parent (P), F1, and F2 generations by examining miR profile in thymocytes. To this end, pregnant mice were injected with DES on GD 14. The thymi of mothers (P), F1, and F2 generations of mice were harvested and high-throughput miR arrays were performed for miR profile. Data obtained from our study showed more than 106 miRs that were common to and dysregulated (upregulated/downregulated) greater than 1.5 fold in thymocytes of all three generations of mice post DES exposure, when compared to controls. miR arrays data post-DES exposure demonstrated changes in miR profile affecting expression of a large number of genes, known to regulate thymic atrophy, cancer, immunosuppression, apoptosis, autophagy, signaling, and other physiological pathways. Also, miR array data showed dysregulation of miRs that may affect various pathways involved in various diseases including cancer, autoimmune diseases, and immune dysfunctions. Together, our studies suggest that DES triggers long-term and transgenerational effects by alterations in miR profile that regulate genes causing immune dysfunction/suppression and long-term effects. Our findings support the concept of fetal basis of adult disease following exposure to DES (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, and P20GM103641 as well as by Veterans Affairs Merit Award BX001357).

3214 Strain Differences in DNA Methylation and Hydroxymethylation Following Prenatal Exposure to Diesel Exhaust Particulate Matter

K. A. Rychlik, J. C. Pulczinski and N. M. Johnson. *Environmental and Occupational Health, Texas A&M University School of Public Health, College Station, TX.*

Early life exposure to particulate air pollution has been associated with a significantly increased risk of offspring asthma development in human studies and experimental models. Evidence supports epigenetic modifications as an underlying mechanism following air pollution exposure during this critical window of susceptibility. These studies were undertaken to evaluate differential DNA methylation following prenatal exposure to diesel exhaust particulate matter (DEPM), a major constituent of air pollution, in two genetically distinct murine strains with established divergent immune responses to DEPM. C57BL/6J and BALB/cJ mice were time-mated and dosed with 50 µg DEPM (NIST, SRM 2975) in PBS or PBS alone via intranasal application every three days throughout gestation. Lung tissues from two-day-old offspring were collected for methylation analysis. Effects on global DNA methylation and hydroxymethylation were analyzed as a first screen to determine differences across treatment group and strain, using an ELISA assay with detection antibodies for 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC). In pups born to DEPM-treated BALB/cJ dams, 5-mC content in DNA from lung tissue was reduced from 2.3% in PBS-controls compared to 1.5% in DEPM-treated. The 5-hmC content was increased from 0.13% to 0.15%, respectively. There was no observable difference in 5-mC or 5-hmC levels in the C57BL/6J treatment groups. These results indicate variation in global DNA methylation and hydroxymethylation in two strains previously shown to exhibit varying susceptibility to DEPM-induced allergic airway disease. This may underlie the heightened response previously observed in BALB/c mice. Current work measuring site-specific DNA methylation and hydroxymethylation is ongoing to further clarify the role of genetic variation on epigenetic reprogramming in response to an important environmental exposure. Additional work is also underway to assess methylation patterns after co-exposure to DEPM and house dust mite, a relevant human allergen, in our chronic model of allergic airway disease.

3215 Baseline Chromatin Modification Levels May Predict Inter-Individual Variability in Ozone-Induced Pro-Inflammatory and Oxidative Stress Gene Expression

S. D. McCullough[1], E. C. Bowers[2], D. M. On[1], D. S. Morgan[1], L. A. Dailey[1], R. N. Hines[1], R. B. Devlin[1] and D. Diaz-Sanchez[1]. *[1]US EPA, Research Triangle Park, NC and [2]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC.*

Traditional toxicological paradigms have relied on factors such as age, genotype, and disease status to explain variability in responsiveness to toxicant exposure; however, these are neither sufficient to faithfully identify differentially responsive individuals nor are they modifiable factors that can be leveraged to mitigate the effects of toxic exposures. Unlike these factors, the epigenome is dynamic and shaped by an individual's environment. We sought to determine whether baseline levels of specific chromatin modifications within the promoters of pro-inflammatory (IL-8, IL-6, and COX2) and oxidative stress (HMOX1) genes correlated with the inter-individual variability in their ozone (O3)-mediated induction in an air-liquid interface model using primary human bronchial epithelial cells from a panel of 11 donors. We characterized the relationship between the baseline abundance of six epigenetic markers with established roles as key regulators of gene expression - trimethyl histone H3 lysine 4 (H3K4me3), acetyl H3K27 (H3K27ac), pan-acetyl H4 (H4ac), di/trimethyl histone H3K27 (H3K27me2/3), unmodified H3, and 5-hydroxymethylcytosine (5-hmC) - and the variability in the O3-induced expression of IL-8, IL-6, COX2, and HMOX1. Baseline levels of H3K4me3, H3K27me2/3, and 5-hmC, but not H3K27ac, H4ac, and total H3, correlated with the inter-individual variability in O3-mediated induction of HMOX1 and COX2. In contrast, none of the chromatin modifications that we examined correlated with the induction of IL-8 and IL-6. From these findings we propose an "epigenetic seed and soil" model in which the baseline abundance of particular chromatin modifications within the regulatory regions of specific toxicant-responsive genes correlates with the magnitude of their exposure-mediated induction. While proposed from data collected from airway epithelial cells, the model may apply to the use of baseline epigenetic data to predict exposure responses in cell and tissue types throughout the body. Understanding the role of the epigenome in toxicant responsiveness could transform our understanding of the mechanisms underlying inter-individual variability in exposure effects and provide new insight into identifying susceptible populations.

3216 *In Vivo* Inhalation Exposures to Jet A and JP-8 Alter Brain miRNA Expression Profiles

J. Frey[1,3], K. Henderson[1,3], K. Mumy[4], C. Gut[2,4], J. Reboulet[2,4], M. Grimm[2,4] and C. Mauzy[1]. *[1]Air Force Research Laboratory, Wright-Patterson Air Force Base, OH; [2]CAMRIS International, Wright-Patterson Air Force Base, OH; [3]Henry M. Jackson Foundation, Wright-Patterson Air Force Base, OH and [4]Naval Medical Research Unit Dayton, Wright-Patterson Air Force Base, OH.*

Jet fuel exposure is a known occupational exposure hazard among military personnel, with exposures initiating neurological symptoms. We hypothesize that microRNA expression changes occur in specific brain regions from repeated exposure to jet fuels, altering gene expression to signaling pathways. Fisher 344 rats were exposed to 4 doses (0, 500, 1000, 2000 mg/m3) of jet fuel administered as an aerosol/vapor combination of either Jet Fuel 8 (JP-8) or Jet A in whole body inhalation chambers. Molecular changes were anchored to phenotype alterations using post-exposure neurobehavioral testing (Morris water maze and acoustic startle reflex). Total RNA was isolated post-exposure from the prefrontal cortex, hippocampus, and cerebellum regions dissected from harvested brain tissue. The isolated RNA samples were analyzed using Affymetrix GeneChip 3.0 miRNA arrays, with the resultant data examined using GeneChip-compatible Expression Console Software v1.2 software (Affymetrix). Differential miRNA expression in the brain was seen between control/JP-8 in prefrontal cortex (miR-429, miR-200a, miR-200b), hippocampus (miR-301a, miR-29c, miR-21), and cerebellum (miR-301a, miR-153, miR-29c). Interestingly, while some overlap in expression alterations was seen, the miRNA signature to Jet A exposure contained unique specific miRNA species: prefrontal cortex (miR-667, miR-328b-3p), hippocampus (miR-352, miR-9, miR-301a, miR-199a-5p, miR-30e), and cerebellum (miR-301a, miR-199a-5p, miR-352). To further explore the biological significance of these molecular changes, pathway analysis was conducted using the Ingenuity IPA software (Qiagen). The result of this study will likely provide novel insight into the molecular mechanism of jet fuel neurotoxicity, as well as the knowledge needed for warfighter protection from the adverse effects of jet fuel exposure.

3217 Changes of Nucleotide Excision Repair (NER) Pathway and Global DNA Methylation Status by Iranian Heavy Crude Oil Exposure: A Toxicological and Epidemiological Study

Y.-H. Kim[3], N. Chatterjee[3], J. Yang[3], M.-S. Park[1], M. Ha[2], H.-K. Cheong[4] and J. Choi[3]. *[1]Taean Institute of Environmental Health Center, Taean, Korea, Republic of; [2]College of Medicine, Department of Preventive Medicine, Dankook University, Cheonan, Korea, Republic of; [3]Environmental Engineering, University of Seoul, Seoul, Korea, Republic of and [4]School of Medicine, Department of Social and Preventive Medicine, Sungkyunkwan University, Suwon, Korea, Republic of.* Sponsor: B.-H. Lee.

A substantial amount of studies conducted on the (eco)toxicity and exposure effects of the Hebei spirit oil spill accident in Taean, South Korea, 2007, but limited in explaining the underlying mechanisms of the same. On one hand, we investigated mechanism of toxicity on the Iranian heavy crude (IHC) oils (the main oil components spilled) using *C. elegans*-Transcription factors (TFs) RNAi library screening. The RNAi-fed worms that showed altered sensitivity in 72 h reproduction test to IHC oils than EV-fed worms were selected. The TFs, T16H12.4 and T24H10.1, were identified as to be involved in protective or in toxic pathway to IHC oil exposure, respectively. With subsequent bioinformatics analysis (KEGG and REACTOME database) nucleotide excision repair (NER) was popped-up which were further confirmed with NER pathway genes (xpc-1, xpa-1, ercc-1, xpg-1, xpd-1 and xpb-1) expressions using qRT-PCR. Increased expressions of xpc-1 and ercc-1 genes, which correspond to damage recognition and TF binding excision, respectively, were observed in IHC exposed wildtype *C.elegans*. As NER pathway is known to be conserved from *C.elegans* to human, further translational study is envisaged on blood samples from people who participated in cleaning after oil spill accident. On the other hand, we examined the global DNA methylation (5-mc) status in blood samples of the participants of oil-cleanup after oil spill accident at 2007. The global DNA hypomethylation (5-mc) was observed in exposed group compared to control and significant DNA hypomethylation trend was also evident with a five year time period (2009 to 2014). Taken together, our preliminary results indicate that global DNA hypomethylation trend and altered NER pathway possibly play significant role in oil spill exposure related diseases.

3218 ssUVR Induces Global Histone Hypoacetylation in Human Hacat Keratinocytes

X. Zhang[2], T. Kluz[2], L. Gesumaria[2], M. Matsui[1], M. Costa[2] and H. Sun[2]. *[1]Estee Lauder Companies, Inc, New York City, NY and [2]Environmental Medicine, New York University School of Medicine, Tuxedo, NY.* Sponsor: M. Costa.

Ultraviolet radiation (UVR) from sunlight is the primary effector of skin DNA damage. Chromatin remodeling and histone post-translational modification (PTM) are critical factors in repairing DNA damage and maintaining genomic integrity, however, the dynamic changes of histone marks in response to solar UVR are not well characterized. To study the possible epigenetic mechanism involved in photoaging and carcinogenesis induced by solar ultraviolet radiation, we analyzed the changes of histone PTMs in immortalized human keratinocytes exposed to solar simulated UVR. A significant decrease in lysine acetylation of histones H3 and H4, particularly at positions of H3 lysine 9, lysine 56, H4 lysine 5, and lysine 16, was found in human keratinocytes exposed to ssUVR. These acetylation changes were highly associated with ssUVR in a dose-dependent and time-specific manner. Interestingly, H4K16ac, a mark that is crucial for higher order chromatin structure, exhibited a persistent reduction by ssUVR that was transmitted through multiple cell divisions. In addition, the enzymatic activities of histone acetyltransferases were significantly reduced in irradiated cells, which may account for decreased global acetylation. Moreover, depletion of histone deacetylase SIRT1 in keratinocytes rescued ssUVR-induced H4K16 hypoacetylation. In summary, our study is the first report indicating that solar UVR can target the activities of both HATs and HDACs and induce global hypoacetylation in multiple sites on Histones 3 and 4. Given the therapeutic potential of HDAC inhibitors, further study of specific enzymes that mediate ssUVR induced epigenetic alteration will provide more insights into future epigenetic therapy.

3219 Gene-Specific 5′-UTR Methylation vs. Promoter Methylation in Leukocytes from Workers Exposed to Different Levels of VOCs

O. A. Jiménez-Garza[2], L. Reynaga-Ornelas[2], A. Dávalos-Pérez[2] and A. Albores[1]. [1]*Department of Toxicology, CINVESTAV, México DF, Mexico and* [2]*Health Sciences Division, Universidad de Guanajuato Campus León, León, Mexico.*

As a result of exposure to toxic substances, different human and animals cells have displayed either global DNA hypomethylation or gene-specific hypermethylation. Specifically in occupational volatile organic compound (VOC) exposure, we previously found hypermethylation in TOP2A, SOD1 and TNF-α promoter area in leukocytes from people exposed to a complex VOC mixture, while participants exposed to a single VOC did not show any differences compared to controls. The aim of this study was to analyze the methylation pattern in the 5′-UTR region, considered for some authors as an "internal/alternative promoter area" in people occupationally exposed to a single VOC compared to VOC mixtures exposure as well as a reference, non-exposed group. Methods: PCR-pyrosequencing was performed in order to analyze methylation status in CpG's located at the 5′-UTR region of the TOP2A, SOD1, IL6, TNF-α and CYP2E1 genes in leukocytes from Mexican workers laboring at three different scenarios with VOC exposure compared to a reference group. Results: 5′-UTR region from IL6 and TOP2A genes showed hypomethylation for two populations exposed to a VOC mixture, while TNF-α showed hypermethylation; methylation in the SOD1 gene did not show differences between groups. DNA obtained from the reference group showed hypermethylation in the 5′-UTR region from the CYP2E1 gene compared to all exposed subgroups. Conclusions: 5′-UTR region in certain genes is more prone to undergo epigenetic modifications compared to promoter area as a result of VOC exposure. 5′-UTR methylation status must be considered in a near future as an early biomarker of effect in human chemical toxicity.

3220 Epigenetic Modifications Are Indicative of the Magnitude and Duration of Ozone-Responsive Gene Expression

E. Bowers[1,2], D. On[2], D. Morgan[2], L. Daily[2], D. Diaz-Sanchez[2] and S. McCullough[2]. [1]*UNC Chapel Hill, Chapel Hill, NC and* [2]*US EPA, Chapel Hill, NC.*

Predicting the health effects of exposure to ozone, a ubiquitous air pollutant, is complicated by the fact that individuals exhibit heterogeneity in inflammatory responses. When exposed to ozone, sensitive individuals express heightened levels of pro-inflammatory and oxidative stress genes that can be orders of magnitude greater than non-responsive individuals. As other studies have not delineated a clear mechanism for this variability, we propose a novel approach of examining the epigenome. We hypothesized that epigenetic regulators of transcription, specifically histone modifications, are responsible for inter-individual differences in the expression of ozone-responsive genes. To investigate this hypothesis, we used primary human bronchial epithelial cells from different donors to characterize coinciding histone modification and expression changes in the ozone responsive genes IL-8, IL-6, COX-2, and HMOX-1. Cells were exposed at air-liquid interface to ozone and harvested for chromatin immunoprecipitation (ChIP)-qPCR and gene expression analysis up to two hours post-exposure. We examined two marks known to activate transcription, trimethylation of lysine 4 on histone H3 (H3K4me3) and acetylated histone H4 (H4Ac) as well as the repressive mark H3K27me3. We observed extensive heterogeneity in histone modification changes following ozone exposure. However, common patterns were observed that were associated with differences in gene expression. For example, increased levels of H3K4me3 one hour post-exposure were associated with a two-fold elevation in IL-6 expression. We also found that elevated activating marks were associated with duration of expression. For example, donors with elevated levels of H3K4me3 and acetylated H4 one or two hours post-exposure had two-fold greater expression of IL-8, HMOX-1, and COX-2 at the two hour time-point. We did not observe any correlations with H3K27me3, suggesting that activating marks may be more important in shaping these responses than repressive marks. These results reinforce our earlier findings that baseline histone modifications may predict ozone responsiveness. Furthermore, they provide a mechanistic insight into inter-individual variability in the ozone-mediated inflammatory response, and broadly, may implicate chromatin modifications as important determinants of toxicant-induced responses.

3221 Hydroxychloroquine Attenuates Cigarette Smoke Induced Autophagic Signaling in the Ovary of Mice

H. C. Furlong[2], M. R. Stämpfli[2], A. M. Gannon[2] and W. G. Foster[2]. [1]*Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada and* [2]*Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada.*

Cigarette Smoke (CS) exposure is associated with accelerated follicle loss, decreased fertility and premature menopause. We previously demonstrated that CS exposure induces autophagy in ovarian granulosa cells in preference to apoptosis and is a potential novel alternative cell death pathway. We recently showed that CS-exposure activates the autophagic cascade through attenuated mTOR expression and enhanced AMPK activation in response to elevated levels of ROS. We postulate that autophagy is a common novel pathway central to impaired follicle development that could explain low dose effects of chemical induced ovarian toxicity and subfertility. The objective of the present study was to determine if chloroquine, a well-known inhibitor of autophagy, could inhibit CS-induced autophagy in the ovary. Eight week old mice were surgically implanted with chloroquine containing pellets; 0, 25, and 50 mg chloroquine/Kg. Half the animals in each treatment group were exposed to room air only and the other half were exposed to CS twice daily for 8 weeks; the equivalent of a pack of cigarettes a day. Ovaries from the mice were harvested at the time of sacrifice for transmission electron microscopy, gene and protein expression analysis. A significantly increased number of autophagosomes and autophagic signalling, evident from the formation of autophagosomes in the granulosa cells, increased expression of autophagic targets LC3, BECN1 and Prkaa2 and decreased expression of pro-survival target BCL-2 was found in the ovaries of CS-exposed mice compared to controls. Furthermore, treatment with chloroquine alone did not show effects distinguishable from the control group. However treatment with chloroquine and CS showed a trend toward attenuation of autophagic signalling as shown by increases in ovary and body weight and decreased expression of the autophagic gene and protein targets relative to CS exposure alone. In conclusion, our results suggest that therapeutic disruption of autophagy is feasible in the ovary and offers promise for ovarian protection from toxic insult and potentially fertility preservation.

3222 Hydrogel Based 3-Dimensional (3D) System for Toxicity and High-Throughput (HTP) Analysis for Cultured Murine Ovarian Follicles

H. Zhou[2], M. A. Malik[2], A. Arab[2], M. T. Hill[2] and A. Shikanov[2]. [1]*College of Literature, Science and the Arts, University of Michigan, Ann Arbor, MI and* [2]*Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI.*

Various toxicants, drugs and their metabolites carry potential ovarian toxicity. Ovarian follicles, the functional unit of the ovary, are susceptible to this type of damage at all stages of their development. However, despite of the large scale of potential negative impacts, assays that study ovarian toxicity are limited. Exposure of cultured ovarian follicles to toxicants of interest served as an important tool for evaluation of toxic effects for decades. Mouse follicles cultured on the bottom of a culture dish continue to serve an important approach for mechanistic studies. In this paper, we demonstrated the usefulness of a hydrogel based 3-dimensional mouse ovarian follicle culture as a tool to study ovarian toxicity in a different setup. The 3D *in vitro* culture, based on fibrin alginate interpenetrating network, preserves the architecture of the ovarian follicle and physiological structure-function relationship. We applied the novel 3D high-throughput (HTP) *in vitro* ovarian follicle culture system to study the ovotoxic effects of an anti-cancer drug, Doxorobucin (DXR). The fibrin component in the system is degraded by plasmin and appears as a clear circle around the encapsulated follicle. The degradation area of the follicle is strongly correlated with follicle survival and growth. To analyze fibrin degradation in a HTP manner, we created a custom MATLAB® code that converts brightfield micrographs of follicles to binary images, followed by image analysis. We did not observe any significant difference between manually processed images to the automated MATLAB® method, thereby confirming that the automated program is suitable to measure fibrin degradation to evaluate follicle health. The cultured follicles were treated with DXR at concentrations ranging from 0.005 nM to 200 nM, corresponding to the therapeutic plasma levels of DXR in patients. Follicles treated with DXR demonstrated decreased survival rate in greater DXR concentrations. We observed partial follicle survival of 35% ± 3% (n=80) in 0.01nM treatment and 48% ± 2% (n=92) in 0.005nM, which we identified as the IC50 for secondary follicles. In summary, we established a 3D *in vitro* ovarian follicle culture system that could be used in an HTP approach to measure toxic effects on ovarian follicles. Zhou et al., PLoS ONE, 2015, in print.

3223 Environmentally Relevant Concentrations of Lead Alter the Late Events of Oocyte Maturation

G. Mosqueda-Tapia, D. G. Acuña-Hernández and I. Hernández-Ochoa. *Department of Toxicology, Cinvestav-IPN, México, DF, Mexico.*

Prior to ovulation, the luteinizing hormone acts on the cumulus cells-oocyte complex (COC) to promote cumulus cells (CC) expansion, and oocyte maturation in which meiosis resumes from profase I to progress to metaphase II (MII) with a concomitant increase in zinc (Zn2+) levels. Lead (Pb2+), a heavy metal used in painting, cosmetics and batteries, has a high affinity for Zn2+ binding sites, and it has been associated with impaired female fertility and decreased number of oocytes resuming meiosis, but the underlying mechanisms remain unknown. This study evaluated the effect of Pb2+ on the oocyte maturation. As mechanisms of toxicity, this study compared the effect of TPEN, a specific intracellular Zn2+ quelator, with those of Pb2+ on parameters such as CC expansion, and stages of meiosis including prometaphase I (PM), metaphase I (MI), anaphase/telophase I (A/TI), and metaphase II. COC's primary cultures from pubertal female CD1 mice were exposed to 0, 0.1, 10, 250, 500 nM of Pb2+, or 10 µM of TPEN. Cell viability was evaluated by staining with propidium Iodide-Hoescht 33342. Stages of meiosis were evaluated by staining DNA with propidium Iodide. Micrographs of COCs were captured at 0 h and 16 h of COC culture, and the diameter of CC expansion was evaluated using the Image Pro-premiere 9.0 software. Our data showed that Pb2+ concentrations did not alter oocyte viability, meiotic resumption nor CC expansion. However, the Pb2+ concentration equivalent to the maximum safe exposure limit (500 nM or 10 µg/dL) significantly decreased the percent of oocytes reaching MII compared to control. Contrastingly, TPEN significantly increased the number of oocytes in A/TI, and decreased the CC expansion. These data suggest that Pb2+ inhibits the last events in oocyte maturation without involving detrimental effects on CC expansion, and through alternative mechanisms from Zn2+ competition or displacement during transition from A/TI to MII. Supported by CONACyT 167678.

3224 Phthalate Mixture Exposure Reduces Antral Follicle Growth and Hormone Production in Cultured Mouse Follicles

C. Zhou and J. A. Flaws. *University of Illinois, Urbana, IL.*

Phthalates are used in a large variety of products, such as building materials, medical devices and personal care products. Most previous studies on phthalates have focused on single phthalates and not mixtures. It is important to study mixtures because humans are exposed to phthalate mixtures on a daily basis. Thus, we tested the hypothesis that exposure to an environmentally relevant phthalate mixture decreases antral follicle growth and hormone production in cultured mouse antral follicles. To test this hypothesis, antral follicles from adult CD-1 mice were cultured with dimethylsulfoxide (DMSO; vehicle control) or phthalate mixture (1–500 µg/ml; n=6 independent cultures, 6–12 antral follicles per treatment group per culture) for 96hours. The phthalate mixture was based on the composition of phthalates detected in urine samples from pregnant women in Illinois. The mixture included 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate. Antral follicle diameters were measured every 24 hours as an indicator of growth. After culture, media were collected and subjected to enzyme-linked immunosorbent assays for measurements of progesterone, androstenedione, testosterone, estradiol, and estrone levels. The results indicate that high doses of phthalate mixture (100 and 500 µg/ml) significantly decrease antral follicle growth, starting at 24 hours (500 µg/ml) and 48 hours (100 µg/ml) of culture ($p<0.01$). Furthermore, exposure to the phthalate mixture significantly decreases androstenedione (10, 100, and 500 µg/ml), testosterone (10 and 500 µg/ml), estradiol (10, 100, and 500 µg/ml), and estrone (100 and 500 µg/ml) levels when compared to control ($p<0.01$). However, exposure to the phthalate mixture does not affect progesterone levels when compared to control ($p>0.05$). These data suggest that exposure to a phthalate mixture significantly inhibits antral follicle growth and several sex steroid hormone levels. Supported by NIH P01 ES022848 (JAF), EPA RD-83459301 (JAF), and an Environmental Toxicology Fellowship (CZ).

3225 Resveratrol Protects the Ovary Against CrVI-Toxicity by Enhancing Endogenous Antioxidant Enzymes and Inhibiting Metabolism of Estradiol

S. K. Banu, J. A. Arosh and R. C. Burghardt. *Veterinary Integrative Biosciences, Texas A&M University, College Station, TX.*

Resveratrol (RVT) (3,4′, 5-trihydroxystilbene), a polyphenolic component in grapes and red wine, has been known for its cytoprotective actions against neurodegeneration, cardiovascular disease, cancer, diabetes, and obesity-related disorders, and extending lifespan of organisms. Chromium (Cr) compounds are used in many industries such as leather tanning, metal plating, and other metallurgical procedures. Increased usage and the inadequate disposal of Cr wastes contributed to the increased levels of Cr in the environment above the safety limits. The safety level of Cr in the drinking water according to the standards of US Environmental Protection Agency (EPA) is 0.1 ppm (100 ppb). However, there are several cities in the US and in developing countries, where the drinking water or the ground water levels of Cr increased to several folds than the approved safety limits. Our recent findings indicate that CrVI exposure during prenatal or postnatal development increased follicle atresia through increased oxidative stress and activation of p53 pathway and apoptosis of oocytes and granulosa cells. Therefore, in the current study, we determined the protective effects of RVT on CrVI-induced ovarian toxicity. Lactating mother rats were treated with potassium dichromate (CrVI, 25, 50, 100 ppm) through drinking water from postpartum day 1-21. On postnatal day (PND)25, F1 female pups were euthanized and ovaries removed and blood was collected. Results indicated that CrVI: (i) increased atresia of primordial and antral follicles; (ii) decreased cell survival pathway (PCNA, HIF1 alpha, BCL2 and BCL XL); (iii) increased cell death pathway (cytochrome C, BAX and caspase 3); (iv) decreased steroids biosynthesis (testosterone (T), estradiol (E2), and progesterone (P4); decreased StAR, 3 beta HSD, 17 beta HSD and cytochrome p450 aromatase; (v) increased estradiol metabolism by enhancing sulphation, hydroxylation and glucuronidation of E2; (vi) decreased FSH receptor and Estrogen receptor (ER) beta); (vii) increased ROS levels in the ovary and plasma; and (viii) decreased cytoplasmic and mitochondrial AOX enzymes. RVT protected the ovary against CrVI-toxicity by enhancing endogenous AOX enzymes, and inhibiting catabolism of E2, thus protecting the ovary against follicle atresia and restoring E2 levels against CrVI toxicity.

3226 Regulation of Ovarian Multidrug Resistance Protein 1 and P-Glycoprotein by Phosphatidylinositol-3 Kinase Signaling

P. Q. Thomas and A. Keating. *Animal Science, Iowa State University, Ames, IA.*

Mammalian females are born with a finite number of oocytes, the female gamete, enclosed in a follicular structure. Upon complete follicle depletion, menopause occurs. Ovotoxicant exposures can cause infertility or hasten the onset of ovarian senescence, referred to as premature ovarian failure. Phosphoramide mustard (PM), the anti-neoplastic and ovotoxic metabolite of cyclophosphamide causes ovarian DNA adduct formation and follicle depletion. Adenosine triphosphate binding cassette (ATP) drug transporters contribute to phase III drug metabolism by binding to conjugated xenobiotics, and contributing to cellular excretion. P-glycoprotein (PGP) transports large hydrophobic compounds and is abundant in liver, kidney, the blood-brain barrier and the blood-testis barrier. Multidrug resistance protein 1 (MRP1) can extrude large organic anionic compounds, hydrophobic compounds, and glutathione conjugates. It also exports sulfonated compounds, such as sulfonated estrogen, thereby contributing to basal ovarian physiological function. There is little information on ovarian phase III drug metabolism. We previously reported increased mRNA encoding Pgp in response to PM exposure, however, no impact of PM on Mrp1 was noted. The PI3K signaling regulates ovarian primordial follicle activation. In addition, we have previously discovered regulation of phase II drug metabolism enzymes by the PI3K pathway. In order to investigate ovarian regulation of MRP1 and PGP, fisher 344 neonatal rat ovaries were exposed to media containing either vehicle control or the PI3K inhibitor LY294002 (20 µM) for 4 days. Total ovarian protein was isolated and western blotting was performed to quantify MRP1 and PGP protein abundance during PI3K inhibition. PGP protein level was decreased ($P < 0.05$) in ovaries lacking PI3K signaling. In addition, MRP1 protein abundance was essentially absent ($P < 0.05$) in LY294002-treated ovaries. In order to determine the ovarian localization of MRP1 and MDR1, ovaries were obtained from adult mice, and immunostaining performed. MRP1 was localized to the oocyte cytoplasm as well as in theca cells. MDR1 was also present in ovarian theca cells, and surprisingly was localized to the oocyte cytoplasmic membrane. These data indicate a critical role for PI3K in regulation of ovarian phase III drug transporters, as well as demonstrate that the oocyte as well as the vascularized thecal cell layer have the capacity to function in phase III drug metabolism.

3227 Gene-environment Interaction and Risk of Preterm Birth with Special Reference to mRNA Expression of IL-4 And IL-10 and Exposure to Orgnochlorine Pesticides

V. Tyagi, B. D. Banerjee, T. Sharma, R. S. Ahmed and K. Guleria. *Department of Biochemistry, University College of Medical Sciences and GTB Hospital, Delhi, India; Department of Obstetrics and Gynaecology, University College of Medical Sciences and GTB Hospital, Delhi, India.*

Preterm birth (PTB) is the major cause of perinatal morbidity and mortality and is the leading cause of neonatal deaths. PTB is hypothesized to be influenced by inflammatory responses. Despite the intensive research, the etiology of idiopathic PTB is still unknown. Organochlorine pesticides (OCPs) are widely used in India under public health program and agriculture. OCPs are xenoestrogenic in nature, slow in degradation and highly lipophilic. These pesticides tend to bio-accumulate in lipid-rich tissues because of strong lipophilic nature and slow biodegradability. Due to a higher percentage of body fat in women, bioaccumulation rate of OCPs is high in women resulting into a greater threat to toxic effects. The present study is designed to explore associations of blood concentrations of OCPs with espression of anti-inflammatory (IL-4 and IL-10) genes in PTB cases. Maternal blood samples of PTB cases and equal number of term delivery as control (n=30 for each group) were collected at the time of spontaneous labor among North Indian population. The study was conducted in the Department of Obstetrics and Gynecology, and Biochemistry at Guru Teg Bahadur Hospital associated with University College of Medical Sciences (University of Delhi), Delhi, India from July 2013 to July 2014. Women who were occupationally exposed to OCPs and other known high-risk factors for PTB or recent intakes of any anti-inflammatory drugs/steroids were excluded from both the groups. The OCP levels were estimated by gas chromatography equipped with ECD detector, and analysis of mRNA expression of IL-4 and IL-10 was carried out using Real-Time quantitative PCR (RT-qPCR). In our study, we have estimated levels of eighteen OCPs out of which significantly higher levels of β-HCH (p=0.001), p'p'-DDE (p=0.003), and o'p'-DDD (p=0.047) were observed in PTB cases as compared to controls. A significant interaction was found between mRNA expression and pesticides levels. Based on our findings, we hypothesized that evaluating the molecular control of inflammation along with gene environment interaction may be used as a model to explore the etiology of idiopathic PTB and may be considered for the prognosis of adverse reproductive outcomes.

3228 Bisphenol A Decreases Gap Junction Channels and Alters Meiosis in Cumulus Cells-Oocyte Complex

D. G. Acuña-Hernández[2], L. Arreola-Mendoza[1], R. Santacruz-Márquez[2], S. P. García-Zepeda[2] and I. Hernández-Ochoa[2]. *[1]Department of Biosciences and Engineering, CIIEMAD-IPN, México, DF, Mexico and [2]Department of Toxicology, Cinvestav-IPN, México, DF, Mexico.*

Oocyte maturation (OM) occurs in preovulatory follicles (PF), specifically in the cumulus cells-oocyte complex (COC), and it is a crucial process for the oocyte fertilizing capability. During OM, luteinizing hormone (LH) stimuli triggers meiosis resumption from prophase I (PI) to metaphase II (MII) and a concurrent cumulus cells (CC) expansion, both favored by the presence of gap junction intercellular communication (GJIC) mainly composed of connexin 43 (Cx43) between CC and the oocyte. Specifically, when LH stimuli starts, CC expansion begins, GJIC are closed, and meiosis resumes to progress to MII. Bisphenol A (BPA), a monomer used in plastics and epoxi resins, has been associated with altered oocyte meiosis progression, impaired fertilizing ability of the oocyte and premature closure of GJIC in somatic cells. However, no studies have related the effect of BPA on CC expansion and alterations in the oocyte meiosis. The aim of this study was to elucidate whether BPA alters oocyte meiosis resumption and progression to MII through a premature blockade of GJIC in COCs. Mouse COC primary cultures were exposed to 2.2, 22, 220 and 2200 nM BPA (2 h/37°C), and then incubated with phorbol myristate acetate (PMA) for 16 h to mimic the LH effect and promote the oocyte maturation. Oocyte viability, stages of meiosis at PI and MII, and loss of GJIC in CC were evaluated. Our results showed that BPA concentrations were safe for the oocyte viability. All BPA concentrations increased meiotic resumption and decreased meiotic progression to MII. BPA at 2.2 and 2200 nM decreased GJIC, evidenced by the gap-FRAP technique, and did not modify Cx43 mRNA levels and Cx43 protein levels. In conclusion, environmentally relevant concentrations of BPA may promote premature meiotic resumption in the oocyte via premature closure of gap junction channels in CC, a mechanism that may contribute to a delayed completion of meiosis. Supported by CONACyT-México 167678.

3229 An Environmentally Relevant Dose of Bisphenol A Decreases Gap Junction Intercellular Communication But Does Not Increases Apoptosis Levels in Cumulus Cells

R. Santacruz-Márquez[1], S. P. García-Zepeda[1], C. A. Mendoza-Rodríguez[2], D. G. Acuña-Hernández[1] and I. Hernández-Ochoa[1]. *[1]Department of Toxicology, Cinvestav-IPN, México, D.F., Mexico and [2]Facultad de Química, Universidad Nacional Autónoma de México, México, D.F., Mexico.*

Prior to ovulation, cumulus cells (CC) maintain a close association with the oocyte through gap junction intercellular communication (GJIC) to facilitate the transfer of factors needed to maintain the function of the female gamete. Bisphenol A (BPA), a plasticizer used in the manufacture of polycarbonate products, has been associated with decreased fertilization ability of oocytes, decreased GJIC in epithelial cells, and apoptosis induction in ovarian cells. However, it is unknown whether the loss of GJIC between CC is related with increased levels of apoptosis, which could be related to the decreased fertilization ability of the oocyte. This study evaluated whether *in vivo* BPA exposure decreases GJIC in CC and induces apoptosis in the cumulus cells-oocyte complex (COC). Adult C57BL/6J female mice were orally dosed with BPA (50 µg/kg bw/day) or vehicle for three estrous cycles. At the end of treatment, female mice received ip 5 IU of equine chorionic gonadotropin (eCG), and 48 h later ovaries were collected to isolate COCs or to be processed for histology. The collected COCs were used either to evaluate GJIC using the Fluorescence Recovery After Photobleaching (FRAP) technique or to evaluate mRNA levels of Bax, a pro-apoptotic factor, and Bcl2, an anti-apoptotic factor. DNA fragmentation in CC and granulosa cells (GC) from preovulatory follicles was assessed in histological ovarian slides by the Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The data showed a significant decreased GJIC between the CC from BPA-treated mice compared to control. However, BPA-treated mice had similar mRNA levels of Bcl2 and Bax in COCs and similar DNA fragmentation in CC and GC compared to control. These data suggest that *in vivo* exposure to an environmentally relevant dose of BPA for three estrous cycles prematurely closes the GJIC between the CC, without adversely altering the basal levels of apoptosis in the CC or CG. Supported by CONACyT 167678.

3230 Bisphenol A Alters Oxidative Stress-Related Factors in the Mouse Ovary

S. Patel and J. A. Flaws. *Comparative Biosciences, The University of Illinois, Urbana, IL.*

Bisphenol A (BPA) is a synthetic estrogen used to impart flexibility in polycarbonate plastics. BPA is also found in many food and beverage containers and can leach from these products over time, thereby potentially exposing humans to BPA. This is of concern because previous studies indicate that BPA exposure can affect reproductive health. Specifically, BPA has been shown to inhibit follicle growth, alter hormone levels, and induce atresia in the mouse ovary. Although the exact mechanism by which BPA exerts these effects remains unknown, studies conducted with other endocrine disrupting chemicals indicate that they inhibit follicle growth and induce atresia through an oxidative stress pathway. Oxidative stress occurs when more reactive oxygen species, such as superoxide and hydrogen peroxide, are present in tissues than the body can detoxify. These reactive oxygen species can cause damage to DNA, RNA, and proteins. To protect against oxidative stress, tissues produce antioxidant enzymes such as superoxide dismutase 1 (SOD1), catalase (CAT), glutathione reductase (GSR), and glutathione peroxidase (GPX). Thus, this study was designed to test the hypothesis that BPA exposure (1 µg/mL to 50 µg/mL) alters the expression of the genes Sod1, Cat, Gsr, and Gpx in mouse antral follicles. To test this hypothesis, antral follicles from adult CD-1 mice were cultured with vehicle control (dimethylsulfoxide, DMSO) or BPA (1, 10, 25, and 50 µg/mL) for 48 and 96h. At the end of each culture period, the antral follicles were subjected to real-time PCR for analysis of antioxidant gene expression. Our results indicate that at 48h, exposure to BPA (10 µg/mL) significantly decreases expression of Sod1 (0.77 + 0.04) compared to DMSO (1.06 + 0.09; n=5, p≤0.05). Additionally, at 48 and 96h, BPA exposure (25 µg/mL) significantly increases Cat expression (48h: 1.58 + 0.15; 96h: 1.96 + 0.20) compared to DMSO (48h: 1.03 + 0.09; 96h: 1.24 + 0.17; n=3-5, p≤0.05). Further, at 48 and 96h, BPA exposure (10 and 25 µg/mL) significantly decreases Gsr expression (48h: 10 µg/mL: 0.88 + 0.05, 25 µg/mL: 0.95 + 0.06; 96h: 10 µg/mL: 0.80 + 0.02, 25 µg/mL: 0.81 + 0.06) compared to DMSO (48h: 1.17 + 0.07; 96h: 1.01 + 0.05; n=3-5, p≤0.05). In contrast, BPA exposure did not affect the expression of Gpx compared to DMSO (n=3-5). From these results, we conclude that BPA alters the expression of antioxidant genes in the ovary, potentially resulting in oxidative stress-related follicle growth inhibition. Support: NIH ES019178.

3231 Aberrant Uterine SIX1 Expression May Promote Uterine Adenocarcinoma Following Neonatal Xenoestrogen Exposure

A. A. Suen[2,3], W. N. Jefferson[3], E. Padilla-Banks[3], V. L. Bae-Jump[2] and C. J. Williams[2,3]. [1]*Curriculum in Toxicology, UNC Chapel Hill, Chapel Hill, NC;* [2]*Lineberger Cancer Center, UNC Chapel Hill, Chapel Hill, NC and* [3]*Reproductive and Developmental Biology Laboratory, National Institute of Environmental Health Sciences, Durham, NC.*

Exposing mice on neonatal days 1-5 to the synthetic estrogen, diethylstilbestrol (DES; 1 mg/kg/day), or the phytoestrogen, genistein (50 mg/kg/day), results in high incidences of uterine adenocarcinoma by 18 months of age. Prepubertal ovariectomy prevents the cancer phenotype suggesting that endogenous estrogen exposure is necessary for cancer promotion. Sine oculis-related homeobox 1 (SIX1) is a cancer-associated transcription factor that is permanently upregulated in the uterus after these exposures. Here we investigate the role of aberrant uterine SIX1 expression in the development of xenoestrogen-induced mouse uterine adenocarcinoma and SIX1 as a biomarker in human endometrial cancer. We found that Six1 mRNA expression was dramatically increased at 6, 12, and 18 months of age in uteri from mice neonatally treated with DES or genistein and positively correlated with uterine adenocarcinoma development. Furthermore, immunohistochemical staining showed that SIX1 protein localized to all uterine hyperplastic and neoplastic lesions in mice neonatally treated with DES or genistein. Prepubertal ovariectomy of neonatally DES treated mice resulted in significantly lower Six1 mRNA expression than in neonatally DES treated intact or adult ovariectomized mice. These findings suggest that uterine SIX1 expression is initiated by neonatal xenoestrogen exposure and perpetuated by endogenous estrogen. In clinical samples of human endometrial cancers, we found a positive correlation between SIX1 expression and endometrial cancer incidence. These findings indicate that uterine SIX1 expression is a disease biomarker and suggest that SIX1 may play a role in uterine carcinogenesis in mice and women. We are currently using mouse genetic models to investigate if SIX1 is necessary or sufficient for cancer development.

3232 Differential Regulation of Human Placental Transporters During Hypoxia

L. Gorczyca. *Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.* Sponsor: L. Aleksunes.

Uptake and efflux transporters expressed in the placenta mediate the maternal-fetal transfer of nutrients, drugs, and environmental chemicals. Compared to atmospheric levels (20% O2), the early placenta develops under hypoxic conditions (3% O2) and by the second trimester the oxygen tension rises to 8% O2. Pathologic conditions such as intrauterine growth restriction are complicated by hypoxia. In this study, we assessed the regulation of placental transporters (BCRP, MDR1, MRP1-3, 5, OATP4A1) in BeWo human choriocarcinoma cells and term human placental explants (n=6) following exposure to 3, 8, and 20% oxygen for 24 h. Incubation of BeWo cells and human explants at 3% and 8% O2 increased mRNAs of two HIF-1 alpha target genes VEGF and GLUT1, confirming activation of this signaling pathway. Compared to the cultures at 20% O2, cells and explants exposed to 3% and 8% O2 exhibited a dose-dependent down-regulation of BCRP mRNA and protein (25-35%) and a 50% reduction of MRP5 mRNA. Conversely, MRP1 mRNA expression was induced 20% in cells and explants in response to hypoxia. Interestingly, while mRNA expression of OATP4A1 was induced by 70% in placental explants, it was down-regulated 60% in BeWo cells under hypoxic conditions. Screening of potential regulators of transporter mRNAs during hypoxia in BeWo cells revealed a significant down-regulation of nuclear receptors PPAR gamma and RXR alpha as well as transcription factors AhR and NRF2. Meanwhile, in response to 3% and 8% O2, the expression of the nuclear receptor ER alpha was induced 6- and 2-fold, respectively. Together, these data suggest that placental expression of transporters is differentially regulated by hypoxia, and that the fetus may be at higher risk of exposure to some xenobiotics early in pregnancy and during fetal diseases when oxygen tension is reduced.

3233 Cholesterol Depletion by Pravastatin Inhibits the Fetoprotective Activity of the Placental Efflux Transporter BCRP

J. T. Szilagyi[4], K. M. Bircsak[4], A. M. Vetrano[3], J. D. Laskin[4] and L. M. Aleksunes[4]. [1]*Environmental & Occupational Medicine, Rutgers University, Piscataway, NJ;* [2]*Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ;* [3]*Neonatology, Robert Wood Johnson Medical School, New Brunswick, NJ and* [4]*Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Pravastatin, a cholesterol synthesis inhibitor that improves endothelial function, is currently in clinical trials for preeclampsia. Statins have been shown to inhibit efflux transporters by depleting plasma membrane cholesterol and disrupting lipid raft structure. Breast cancer resistance protein (BCRP/ABCG2) is an efflux transporter in the placenta that limits fetal exposure to drugs and toxins by driving them back into maternal circulation. Earlier studies suggest that BCRP localizes in lipid rafts, a glycoprotein and cholesterol rich microdomain of plasma membranes. In the present studies, we analyzed BCRP protein enrichment and activity in lipid rafts isolated by density gradient centrifugation using BCRP-overexpressing cells, the human placental BeWo cell line, and in healthy, term human placentas. In all 3 experimental models, western blotting showed the localization of BCRP protein (72 kDa) in lipid raft fractions. In BeWo cells, depletion of cellular cholesterol by treatment with 5 mM methyl β cyclodextrin for 1 hr or 200 μM pravastatin for 48 hr, decreased BCRP efflux activity by ~50%, as measured using the BCRP substrates Hoescht 33342 and zearalenone, an estrogenic mycotoxin that has been shown to effect reproduction after *in utero* exposure. Neither methyl β cyclodextrin nor pravastatin altered expression of BCRP protein in BeWo cells. Repletion of cells with exogenous cholesterol (2 mM) following pravastatin or methyl β cyclodextrin treatment restored efflux transporter activity. Taken together, these data demonstrate that BCRP is contained in lipid rafts and that modulation of cholesterol content disrupts its activity. Clinical use of pravastatin in preeclamptic patients may reduce BCRP functioning in the placental barrier leading to the excessive transfer of drugs and toxins across the placenta and compromise fetal health. (Supported by R01ES020522, T32ES007148, P30ES005022).

3234 Identification of *ABCB1* Promoter Haplotypes and Their Effects on Placental P-gp Expression

J. T. Speidel[2], M. Xu[2] and S. Z. Abdel-Rahman[2]. [1]*Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX and* [2]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX.*

During gestation, the fetus is exposed to endogenous and exogenous compounds including pharmaceuticals, estrogens and environmental toxins. *In utero* exposure to these agents can lead to developmental and behavioral problems and diseases including cancer. To reduce fetal exposure, placental efflux transporters actively extrude xenobiotics from the placenta back to maternal circulation. P-glycoprotein (P-gp), encoded by the ABCB1 gene, is a major efflux transporter in the placenta. Single nucleotide polymorphisms (SNPs) in the ABCB1 promoter region, acting through multiple mechanisms, alter ABCB1 expression and subsequent P-gp protein concentration, ultimately affecting fetal exposure to xenobiotics. Because SNPs typically occur in combinations forming specific haplotypes, they do not exert their effects individually. Haplotypes effect on the transcriptional and translational regulation of the ABCB1 promoter is not fully elucidated, leading to our central hypothesis that promoter haplotypes, rather than individual SNPs, alter ABCB1 transcription regulation and subsequently, placental P-gp levels. To test this hypothesis, we sequenced 100 placental DNA samples to determine the ABCB1 promoter haplotype structure. Within the sample population, 12 SNPs were identified in the ~2500bp promoter region. Using PHASE, 25 haplotypes were inferred ranging in frequency from 0.89 for the ancestral haplotype to 0.00019 for a theoretical haplotype. Further analysis shows that these haplotypes are found as 13 paired combinations. Comparing placental P-gp with these combinations shows a dramatic alteration in P-gp expression ranging from a 2-fold increase (0.33μg P-gp/10μg total protein) to a 4-fold decrease (0.04μg P-gp/10μg total protein) in expression versus the ancestral haplotype (0.16μg P-gp/10μg total protein). However, due to sample size limitations and the low frequency of some haplotypes, the differences were not statistically significant. Currently, we are identifying the haplotype specific induction/repression of ABCB1 when exposed to xenobiotics. The long term goal is to define the underlying mechanisms to transcriptional regulation and to develop novel biomarkers that can help individualize treatment for a pregnant patient. (Supported by T32ES007254, P30 ES006676 and JSME bridging grants)

3235 Interindividual Regulation of the BCRP/ABCG2 Transporter in Human Term Placentas

K. M. Bircsak[4], N. Memon[2], B. I. Weiberger[1], A. M. Vetrano[2] and L. M. Aleksunes[4]. *[1]Hofstra North Shore-LIJ School of Medicine, New Hyde Park, NY; [2]Dept of Pediatrics, Rutgers University, New Brunswick, NJ; [3]Dept of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ and [4]Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.*

The "blood-placenta barrier" contains efflux transporters including the breast cancer resistance protein (BCRP/ABCG2) that limits the placental passage of potentially toxic chemicals. To investigate the interindividual variation in the placental expression of BCRP, 109 term placentas were collected from healthy mothers with uncomplicated pregnancies. Placental mRNA and BCRP protein expression were measured using multiplex branched DNA and western blot analysis, respectively. Placental ABCG2 genotypes were determined using Fluidigm Biomark genetic analysis. Infant gender, ethnicity, transcription factors, and genotype were assessed as contributors to a 6-fold interindividual variability in BCRP mRNA levels observed in this study. Placentas were obtained from an ethnically diverse population including Caucasian (32%), African American (16%), Asian (16%), Hispanic (16%) and mixed backgrounds (20%). No relationship between gender of the infant and BCRP mRNA expression was observed. The mRNA expression of BCRP correlated with two known upstream transcription factors NRF2 (r2= 0.29) and AhR (r2= 0.26). Furthermore, single nucleotide polymorphisms in the 5'UTR (A-34763C) or second intron region (A238G) of the ABCG2 gene were associated with a 50% reduction in BCRP mRNA expression in placentas from Asian women. Interestingly, the BCRP non-synonymous polymorphism (C421A, Q141K) was not associated with altered BCRP mRNA levels, but did correspond with a 50 to 60% decrease in BCRP protein in placentas carrying one or two copies of the variant. Results from this study advance our understanding of the constitutive regulation of placental BCRP expression and may help to identify infants at risk for increased fetal exposure to chemicals that are BCRP substrates. Supported by ES020522, ES005022, ES007148, AFPE and PhRMA Predoctoral Fellowships.

3236 Gene Expression Profiling of the Effects BDE-47 on Human Primary Cytotrophoblasts

J. F. Robinson, M. Kapidzic, E. G. Hamilton, M. J. Gormley, T. Dent, M. T. McMaster and S. J. Fisher. *Department of Obstetrics, Gynecology & Reproductive Sciences, University of California, San Francisco, San Francisco, CA.*

Polybrominated diphenyl ethers (PBDEs) are found in human fetal tissues, including the placenta, with unknown consequences. During human pregnancy, placental cytotrophoblasts (CTBs) play critical roles in uterine invasion, maternal vascular remodeling, and anchoring of the feto-placental unit. In culture, primary human CTBs display cell and molecular behaviors relevant to their *in vivo* functions. Therefore, in this study, we used CTBs from 2nd trimester human placentas as an *in vitro* model to study the effects of two common congeners, BDE-47 and -99, on placental development. First, we investigated the actions of PBDEs on CTB viability, death and migration. After 24h of exposure with either congener, we observed significant, dose-dependent changes in cell viability and death (LDH activity; $p<0.05$). Additionally, cell migration was significantly reduced ($p<0.05$). Next, we studied the effects of a sub-cytotoxic concentration of BDE-47 (1μM) on the transcriptome following a 24h exposure initiated at either 3 or 15h in culture. In total, 675 genes were differentially expressed (DE) due to exposure (ANOVA, $p<0.05$; FC>1.2). In general, the effects on gene expression were independent of the exposure interval. Functional enrichment analyses of DE genes suggested specific pathways were disrupted by BDE-47, including, steroid metabolism, morphogenesis, inflammatory and differentiation. Validation studies, via qRT-PCR, confirmed dose-dependent, altered expression of targets suspected or proven to be involved in CTB differentiation and/or placental development, including GREM1, MMP1, PLAC4, HMGCS1 and FABP7 (ANOVA, $p<0.05$). Our results suggested that PBDEs induced developmental toxicity and molecular perturbations relevant to placental development. Ongoing studies aim to link transcriptomic alterations by PBDEs with functional consequences (i.e., reduced CTB migration, Δ differentiation).

3237 *In Utero* Diesel Exhaust Exposure Induces Altered Placental Vascularization, Placental Nanoparticles Transfer and Intergenerational Effects

S. A. Valentino[3,6], A. Tarrade[3,6], J. Aioun[3,6], E. Mourier[3,6], C. Richard[3,6], M. Dahirel[3,6], D. Rousseau-Ralliard[3,6], M.-C. Aubrière[3,6], S. Camous[3,6], M. Guinot[3,6], M.-S. Lallemand[3,6], M. Charlier[6], E. Aujean[6], H. Al Adhami[3,6], P. H. Fokkens[1] , L. Agier[4], J. A. Boere[1], F. R. Cassee[1,2], R. Slama[4] and P. Chavatte-Palmer[3,6]. *[1]Centre for Sustainability, Environment and Health, National Institute for Public Health and the Environment, Bilthoven, Netherlands; [2]Institute of Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands; [3]PremUp Foundation, Paris, France; [4]U823, IAB Research Center, -Team of Environmental Epidemiology Applied to Reproduction and Respirato, Inserm and Univ. Grenoble Alpes, Grenoble, France; [5]UMR 1198 Biologie du Développement et Reproduction, INRA, Jouy-en-Josas, France and [6]UMR1313 Génétique Animale et Biologie Intégrative, INRA, Jouy-en-Josas, France.* Sponsor: F. Cassee.

Gestational exposure to airborne pollution, in particular diesel engine exhaust (DE), was shown to reduce birth weight, depending on exposure time and duration and nanoparticle (NP) concentrations. Our objective was to evaluate the effects of gestational exposure to DE on feto-placental development over 2 generations. Pregnant female rabbits (F0) were exposed to diluted and filtered DE (N=7) (1mg/m3, mean NP diameter ≈ 69nm) or clean air (N=5) for 2h/d, 5d/wk by nose-only exposure. Feto-placental development was monitored by ultrasound. Dams were euthanized at d28. Placentas were studied by stereology and TEM. Clinical biochemistry was performed on maternal and fetal blood. Growth retardation was observed at 14d with decreased head length ($p=0.04$) and umbilical pulse frequency ($p=0.018$). At 28d, fetal head length ($p=0.029$) and plasma insulin ($p=0.05$) were reduced. Placental efficiency ($p=0.049$), placental blood flow ($p=0.009$) and fetal vessel volume ($p=0.002$) were also decreased. Non-aggregated and "fingerprint" NP were observed in placenta and fetus, indicating transplacental transfer. Adult female offspring from another group were bred with control males. Fetoplacental development was not affected but fetal metabolism was modified in F2 fetuses with decreased cholesterolemia ($p=0.008$) and increased plasma triglycerides ($p=0.015$). In conclusion, repeated daily gestational exposure to DE at levels close to urban pollution can affect feto-placental development in two generations.

3238 *In Vivo* Microvascular Implications of Nano-TiO2 Inhalation Throughout Estrous and During Pregnancy

P. A. Stapleton, C. R. McBride, J. Yi and T. R. Nurkiewicz. *Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV.*

Despite the significant impact on future generations, our understanding of the impact of engineered nanomaterial exposure on the uterine microcirculation in either pregnant or non-pregnant states remains quite limited. While the cardiovascular and endothelial cell dysfunctions are commonly presented using *in vitro* techniques, we have opted to develop intravital microscopy techniques that preserve *in vivo* neurogenic, humoral, and myogenic control while investigating local microvascular activity. Female Sprague Dawley rats were exposed to nano-TiO2 aerosols (10±0.6 mg/m3, 4 hours, aerodynamic diameter of 170±5 nm) leading to a calculated pulmonary deposition of 43±2 μg and stratified based on estrous cycle status (estrus, metestrus, diestrus, proestrus, and full-term [GD 20] pregnancy). 24h post-exposure rats were anesthetized (Inactin, 100 mg/kg, i.p.). The right horn of the uterus was exteriorized, gently secured over an optical pedestal at its *in situ* length, and enclosed in a warmed PSS tissue bath. After equilibration, leukocyte adhesion and rolling were assessed using a 20x water immersion objective on 3 separate venules to characterize leukocyte trafficking in each estrus state after nano-TiO2 exposure. Microvascular reactivity was measured using ionophoretically applied acetylcholine (0.025 M; ACh; 20, 40, 100, and 200 nA), sodium nitroprusside (0.05 M; SNP; 20, 40, and 100 nA), or phenylephrine (0.05 M; PE; 20, 40, and 100 nA) conducted using glass micropipettes to evaluate endothelium-dependent dilation, -independent dilation, and vascular smooth muscle responsiveness, respectively. Passive diameter was established by superfusing the tissue with 10-4M adenosine. Overall, we report disparate results between the control and nano-TiO2 exposure animals. Throughout estrous, leukocyte rolling and adhesion was significantly elevated (2.67 fold change; $p<0.05$)

within uterine venules after nano-TiO2 exposure. Given the complexities of female reproduction, this *in vivo* application will significantly aid in directly revealing some of the microvascular and reproductive ramifications (both pre-conceptive and gestational) associated with female nanomaterial exposure. NIH-K99-ES024783 (PAS); R01-ES015022 (TRN)

3239 Assessing Systemic Toxicity and Therapeutic Efficacy of Atosiban and Nafarelin in a Rabbit Model of Endometriosis

R. Rajoria, J. Leong, C. Smith and V. Naageshwaran. *Absorption Systems, San Diego, CA.* Sponsor: C. Davenport.

Purpose: Endometriosis is a chronic, painful disease whose etiology remains unknown and affects roughly 6–10% of women. Treatment of endometriosis can require laparoscopic removal of lesions and long term pharmacological management of pain and infertility symptoms. To better understand the mechanisms and therapies of this enigmatic disease, an animal model is required to evaluate therapeutics that can help mitigate clinical signs. The goal of an autologous rabbit model of surgically-induced endometriosis is to mimic the disease in women, and determine therapeutic efficacy and potential toxicity of commonly used hormone therapies such as Atosiban (Oxytocin receptor blocking agent) and Nafarelin (Gonadotropin releasing hormone agonist). Methods: A reproducible method of inducing endometriosis was established by excising endometrial tissue and placing it within the abdominal cavity adhered to the serosa of the reproductive tract of New Zealand White rabbits. Confirmation of endometriosis development was determined within 2-4 weeks by: (1) Clinical signs; (2) Intra-abdominal gross tissue evaluation; (3) Ultrasound imaging. Upon confirmation of induced disease, various doses of Atosiban and Nafarelin were administered at pre-selected time-points. In addition, a cohort of rabbits were given doses of each drug prior to surgical induction of disease in order to determine efficacy of drugs in preventing endometriosis. At the end of the treatment regimen clinical pathology, gross necropsy with foci-adhesion measurements, and histopathology of affected tissue were used to assess safety and efficacy of the aforementioned drugs. Results: Endometriosis was detected in all groups with regression of disease seen at certain selected drug doses of Atosiban and Nafarelin without clinical signs of toxicity. Conclusions: As a known induced ovulator, rabbits have been historically used in the field of comparative medicine for studying reproductive disorders. As our study indicated, the formation of endometrial foci in rabbits has proven to be effective in evaluating drug therapies for endometriosis. Although clinical trials have utilized Atosiban and Nafarelin for treatment of clinical signs of this disease, the utilization of these drugs in prevention of disease onset is yet to be determined. Further evidence is required to establish the long term prophylactic benefits of such drugs by determining optimal dosages while minimizing systemic toxicity.

3240 Serum from Pregnant Women Induces Xenobiotic Metabolizing Enzymes and Transporters in Cultured Human Intestinal Cells

J. E. Moscovitz[3], G. Herrera-Garcia[1], G. L. Guo[3] and L. M. Aleksunes[3]. [1]*Dept of Maternal Fetal Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ;* [2]*Dept of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ and* [3]*Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.*

The intestine expresses many critical xenobiotic metabolizing enzymes and transporters that regulate oral absorption and fecal excretion. Important nutrients and vitamins are absorbed at this interface, and as a result expression of these enzymes and transporters are tightly regulated. While it is known that gastrointestinal motility decreases during pregnancy, additional molecular changes in intestinal cells have yet to be investigated. To determine whether pregnancy alters intestinal disposition pathways, RNA and protein were collected from human colon adenocarcinoma LS174T cells cultured in media supplemented with 20% serum from either non-pregnant or pregnant women (weeks 31 to 39 of gestation). In intestinal cells treated with pregnant serum, the mRNA expression of the CYP3A4 enzyme, basolateral efflux transporters OSTα and MRP3, as well as the luminal efflux transporters MDR1, MRP2 and BCRP are induced 2- to 4-fold as compared to cells treated with non-pregnant serum. MRP3 protein exhibits a 2-fold up-regulation when treated with pregnant serum. Profiling of differentially expressed genes (induction of CYP3A4 and MDR1 along with down-regulation of the SHP transcription factor) suggests that serum from pregnant women is activating the Pregnane X Receptor (PXR). Further mechanistic studies reveal that progesterone and its sulfated metabolite PM5S may be responsible, in part, for expression changes observed in cells treated with pregnant serum. Because MDR1, MRP2 and BCRP are important to the luminal efflux of endocrine disrupting chemicals, such as mycotoxins, perfluorinated chemicals, and pesticides, activation of intestinal PXR by hormones in pregnant serum may be an adaptive response to decrease systemic absorption and in turn, fetal exposure. Supported by F31HD082965, R01ES020522, T32ES007148, P30ES005022.

3241 Body Burden and Hepatic Cyp Expression in Rats Following Low Dose Drinking Water Exposure to a VOC Mixture During Pregnancy and Adolescence

A. J. Filgo[3], D. M. Chambers[2], E. M. Quist[1,3], B. C. Blount[2] and S. E. Fenton[3]. [1]*Comparative Biolmedical Sciences, NCSU, Raleigh, NC;* [2]*National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA and* [3]*NTP Laboratory, NIEHS, Research Triangle Park, NC.*

Volatile organic compounds (VOCs) such as benzene, trichloroethylene (TCE), trans-1,2, dichloroethylene (t-1,2-DCE), tetrachloroethylene, and vinyl chloride are common industrial solvents used in dry cleaning and degreasing processes. High cumulative exposures to VOCs at Camp Lejeune were associated with earlier age at onset for male breast cancer. Here, time-pregnant Harlan Sprague Dawley rats (n=2) were gavaged on gestation day (GD)15 at 7 time points over 24 hours with a concentration of VOCs 100X higher than levels detected in contaminated US drinking water, to determine the half-life of the chemicals as a mixture in a pregnant rat. Another group of time-pregnant rats and their offspring (n=2-3) were given access to water containing mixtures of these 5 VOCs at concentrations 5X, 10X and 50X those detected in contaminated drinking water. Dams and pups were exposed from GD10 until sacrifice. Blood was collected under hermetic conditions to determine VOC body burden in both dams (GD13, 15, 20 and postnatal day (PND)15, 21) and the resulting offspring (PND15, 21, 28, 48). In exposed dams, low levels of VOCs were detected in the blood during pregnancy and increased during lactation. Percent dose and percent daily dose were estimated in the dams so that comparisons of VOC found in the blood could be made. The percent dose in blood was less than 1% for all chemicals after one day of exposure and did not accumulate over time. Benzene was detected at higher percent dose than TCE and t-1,2-DCE despite having similar half-lives in the blood (t1/2= 1.61, 1.99 and 1.52 hours for benzene, TCE and t-1,2-DCE, respectively). Offspring VOC levels varied with changing body weight, and milk and water consumption, but did not accumulate. Liver mRNA levels for Cyp2e1 and Cyp2b1 in the offspring did not indicate a change in expression due to VOC exposure; activation assays are needed to confirm these findings. Morphological changes in the mammary gland were detected in the offspring in all dose groups and were most prominent in males. These data suggest that the mammary gland is sensitive tissue to very low levels of VOCs in drinking water and may not be dependent on Cyp enzyme activation in the liver.

3242 Incorporation of Measures for Monitoring an Assessment of the Female Reproductive System in a 52-Week Toxicity Study in the Minipig

A. Makin[1], K. Kaaber[1], M. L. Jensen[1], G. Jeppesen[1], U. Westrup[3], S. Dyring Jakobsen[2] and S. Møller Laursen[2]. [1]*CiToxLAB Denmark, Lille Skenved, Denmark;* [2]*LEO Pharma, 2750 Ballerup, Denmark and* [3]*Westrup Vet. Consulting, 2820 Gentofte, Denmark.*

We have performed a 52-week toxicity study in minipigs by daily oral gavage dosing. The female reproductive system was identified as a potential target for toxicity of the drug in earlier toxicology studies in the rat. In the present study, it was decided to incorporate a number of observations and measurements to assess the reproductive system in the females in order to identify potential signals for toxicity as early as possible. The purpose of including these measurements was to avoid the need to sacrifice groups of animals periodically during the study in order to assess the relevant organs macroscopically and microscopically. The evaluations were: evaluation of the estrus cycle, regular blood sampling for progesterone, periodic (every 3 months) ultrasound scanning of the ovaries and post-mortem evaluation (macroscopic, organ weight and microscopic changes). Estrus cycle evaluation involved a daily observation of the condition of the vulva together with physical signs of readiness to mate (female minipigs will only mate during ovulation). We gained much useful information concerning measurement and observation of the reproductive behaviour of the animals. Normal cycling (estrus cycle length of ca 19 to 20 days) was seen in all but 1 animal which was found, both by ultrasound scanning and by post-mortem examination, to have ovarian cysts. Ultrasound scanning was useful to monitor the normal size and morphology of the ovaries in-life, and was able to identify those animals that were shown to have abnormalities post-mortem.

The battery of tests that we incorporated into this study could be useful to detect any possible changes in the female reproductive system in minipigs during the course of a study. One of the main benefits was to avoid the use of interim kills to detect the timing of potential changes in ovarian morphology.

3243 A Qualification Intravenous Infusion Study in Juvenile Sprague-Dawley Rats

R. Tavcar[2], C. Gordon[2], A. S. Galicia[2], M. S. Maghezzi[2], R. Forster[1] and S. Authier[2]. *[1]CiToxLAB France, BP 563, France and [2]CiToxLAB North America, H7V 4B3 Laval, QC, Canada.*

The ability to administer drugs intravenously to neonatal rats is critical for the assessment of non-clinical development of some pharmaceuticals. The objective of this study was to assess the tolerance of continuous intravenous infusion of saline via a surgically implanted catheter following surgical procedures to neonatal Sprague Dawley rats starting on Day 21 post partum for 35 days. Animals underwent surgery on Day 21 post partum and a medical grade catheter was surgically implanted so the tip of the catheter was located in the vena cava at approximately the level of the kidneys. The catheter was secured in place and exteriorized at the nape of the neck through a subcutaneous tunnel from the inguinal area to the dorso-cervical area. Following recovery from surgery, the animals were placed into 2 groups of 10 animals per/sex with a third (non-operated) group consisting of 5 animals/sex used as controls. Animals received 0.9% saline at a rate of 0.2 or 0.4 mL/hour via an infusion pump. Data were collected on mortality, clinical signs, body weights, food intake, clinical pathology and histopathology. Food consumption and body weight were reduced moderately for pups that underwent surgical procedures in comparison to the controls. Preliminary results obtained from this study provided sufficient evidence that the surgical implanted catheter to weanling rats on Day 21 post-partum was well tolerated at both dose rates and did not results in any unexpected background changes normally observed with surgically implanted catheters.

3244 The Effects of Oral and Dermal Exposure of 1,4-Dinitrobenzene on Oxidative Stress and Sperm Characteristics Leads to Reproductive Toxicity in Male Wistar Rats

J. O. Sangodele and A. C. Akinmoladun. *Biochemistry, Federal University of Technology Akure, Akure, Nigeria.*

1,4-Dinitrobenzene (DNB) a synthetic substance used in making dyes, organic chemicals and plastics. Oral exposure is the most likely route for people living near ammunition plants and dermal exposure for industrial workers. This study investigated the influence of (DNB) on the testicular and epididymal antioxidant systems, sperm parameters and histopathological changes. Oral exposure of rats 50 and 75 mg/kg body weight (bwt) and dermal exposure 1000, 2000 mg/kg bwt to (DNB) for 14 days showed that the testicular antioxidant status at 50, 75, 1000 and 2000 mg/kg bwt and the epididymal antioxidant status were affected compared with control. However, increased hydrogen peroxide and lipid peroxidation levels were accompanied by a concomitant decrease in superoxide dismutase and catalase activities in testes and spermatozoon of rats administered with (DNB). Moreover, (DNB)-treated rats showed decrease in the body, testis and epididymis weights. Epididymal degeneration with concomitant decrease in sperm quantity and quality was observed in rats treated with 50, 75, 1000 and 2000 mg/kg compared with control. The seminiferous tubules appear fibrotic, mildly atrophied with degenerated germinal cells. The spermatogonia cell, sertoli cells and leydig cells appear abnormal in testes. Overall, induction of oxidative stress in the epididymis and testes, could cause reproductive deficits in individuals unduly exposed to 1,4-dinitrobenzene.

3245 Quercetin and Vitamin E Attenuate Bonny Light Crude Oil-Induced Alteration in Testicular Apoptosis, Stress Proteins and Steroidogenic Acute Regulatory Protein in Wistar Rats

A. P. Ebokaiwe. *Chemistry/Biochemistry/Molecular biology, Federal University Ndufu-Alike Ikwo, Abakaliki, Nigeria.*

Studies have shown the reproductive toxicity of Bonny-light crude oil (BLCO) via mechanism of oxidative stress and testicular-apoptosis. Protective role of quercetin and vitamin E on BLCO induced testicular apoptosis was evaluated. Experimental rats were divided into four groups of four each. Animals were orally administered 2mg/kg corn oil, BLCO- 800 mg/kg body weight + 10mg/kg quercetin, BLCO-800 mg/kg body weight + 50mg/kg vitamin E and BLCO-800mg/kg body weight only. Protein levels of caspase 3, FasL, NF-kB, StAR protein and stress response proteins were determined by SDS PAGE. Immunofluorescence staining was used to quantify the expression of caspase 3, FasL and NF-kB. DNA damage was quantified by TUNEL assay. Administration of BLCO resulted in a significant increase in the levels of stress response proteins and apoptotis-related proteins by 50% and above after 7 days following BLCO exposure and a concomitant increase in expression of caspase 3, FasL and NF-kB expression by immunofluorescence staining. The DNA damage showed significant increase in the TUNEL positive cells. Co-administration with quercetin or vitamin E reversed BLCO-induced apoptosis and levels of stress protein, relative to control. These findings reveal Quercetin and vitamin E chemo-protection against BLCO-induced testicular oxidative stress related apoptosis.

3246 Cyp1b1 Is Required for Transplacental Polycyclic Aromatic Hydrocarbon Dibenzo(def,p)chrysene Induced Testicular Damage

E. Madeen[3], T. Harper[3], L. Shorey[3], J. Lim[1], C. Loehr[3], D. Williams[3] and U. Luderer[1]. *[1]Reproductive Medicine, Universtiy of California Irvine, Irvine, CA; [2]Toxicology, Oregon State University, Corvallis, OR and [3]Vetrinary Medicine, Oregon State University, Corvallis, OR.*

Dietary PAH exposure accounts for up to 70% of all PAH exposure and over 90% of exposure to the high molecular weight PAHs, such as DBC and benzo[a]pyrene (BaP), in non-smokers. DBC is genotoxic and can cross the placental barrier. Utilizing Cyp1b1 null and Cyp1b1 wild-type mice, we hypothesize that a maternal gavage of DBC (15, 12, 6.5, and 0 mg/kg) on gestation day (GD) 17 will result in reproductive toxicity in the male F1 generation through oxidative stress via the cytochrome P450 (CYP) 1B1 metabolic pathway. Additionally, a maternal diet rich in the anti-oxidant phytochemical indole-3-carbinol (I3C; 500 ppm) may reduce DBC oxidative damage. Cyp1b1 null F1 weanlings had no reproductive toxicity while cyp1b1 wild-type cohorts had severe toxicity. At 21 days postpartum, the exposed male F1 generation had reduced testes area ($p<0.001$) of 9.3 ± 2 mm2 in 6.5mg/kg mice relative to 20.5 ± 1.6 mm2 in control mice, reduced tubule counts ($p<0.01$) of 115 ± 22 tubules/testis in 6.5 mg/kg mice relative to 187 ± 7 tubules/ testis in control mice, and tubules depleted of spermatocytes 96 ± 4% depletion in 15mg/kg treated mice relative to no depletion in control mice. I3C supplementation insignificantly prevented reduction in the size, spermatocyte depletion, and tubule count in weanlings. Mice assessed at 10 months of age, following GD 17 transplacental exposure had similar results to the 21 day old cohort. Together these data indicate that a single *in utero* exposure to DBC at GD17 results in reproductive toxicity in wild type male F1 mice and that these effects are dependent upon Cyp1b1 bioactivation of DBC as null mice are not susceptible to gonad toxicity.

3247 A Novel *In Vitro* Spermatogenesis Model Demonstrates Reproductive Toxicity of Flame Retardants

A. N. Steves[1], D. Clarkson-Townsend[2], J. M. Bradner[2], W. M. Caudle[2] and C. A. Easley[1]. *[1]Cell Biology, Emory University School of Medicine, Atlanta, GA and [2]Environmental Health, Emory University Rollins School of Public Health, Atlanta, GA.*

ABSTRACT. Infertility is a serious global phenomenon, with approximately 20% of couples worldwide experiencing some form of infertility. Male infertility accounts for about 50% of these cases, but more than 30% of male infertility is classified as idiopathic, or without a known root cause. Environmental exposures may play a significant role in male infertility, but to date, methods to investigate effects of exposure on human male fertility have been limited. We recently developed the first *in vitro* model of human spermatogenesis using human pluripotent stem cells and demonstrated that this model could be used to recapitulate clinical phenotypes seen in human cohorts occupationally exposed to DBCP or 2-BP. Here we use this model to screen two flame retardants TDCPP and HBCD for reproductive toxicity. Flame retardants are widely used in construction materials, furniture, plastics, electronics equipment, textiles, and other materials. These compounds persist in the environment and bioaccumulate in the human body. Evidence for adverse health effects includes endocrine disruption and altered reproductive capabilities, but until now, no model has existed to directly test their direct effects on human spermatogenesis. Our data show that acute exposure to TDCPP activates caspases and induces apoptosis in spermatogenic cells, increases cell cycle arrest at G0/G1, and decreases the viability of PLZF+ spermatogonia. Similarly, acute treatment with HBCD increases cell death but does not affect the cell cycle. Future work involves completing these assessments with TBBPA, TBPH, and TBB as well as examining germ cell gene expression and global DNA methylation and

parent-of-origin imprints on haploid spermatids from exposed cultures. Based on these results, our model may be the first *in vitro* human model available to screen for male reproductive toxicants and potentially explain male factor infertility caused by environmental exposures to various chemicals such as flame retardants.

3248 Relevant Methods of Oral Exposure to E-Waste Leachate and Fracking Fluid Alters Reproductive Sperm Parameters in Male Mice

P. H. Costa[1], J. Igbo[2], J. Blum[1], M. Sisco[1], S. Doherty-Lyons[1], K. Kaur[1], M. McCawley[3] and J. Zelikoff[1]. [1]*Dept of Environmental Medicine, NYU School of Medicine, New York, NY;* [2]*Environmental Medicine, Dept of Biological Oceanography Nigerian Institute of Oceanography and Marine Research, Lagos, Nigeria and* [3]*School of Public Health, West Virginia U, Morgantown, WV.*

The potential human harm from unregulated manual disassembly of electronic waste (E-waste) or fracking fluid spills could arise from exposure in soil, rivers, groundwater, and vegetation near residential sites. Both wastewater types contain chemical mixtures with the potential to adversely impact reproductive health if ingested. Thus, a laboratory study was carried out with adult B6C3F1 male mice to evaluate the persistent toxicity of e-waste leachate and fracking-produced wastewater on male reproductive development. Three groups (n=15) of 8-wk-old mice were exposed (5 d/wk/5 wk) by gavage (0.25ml/mouse) to either e-waste leachate from the Alaba Market (Lagos, Nigeria) or, via a novel mealworm (*Tenebrio molitor*) feeding route (0.25ml/2 worms) to W. Virginia fracking wastewater or deionized water. Mice were sacrificed 24 hr, 3 wk or 5 wk post-exposure, and testes, cauda epididymis, seminal vesicles, and prostate were collected and weighed. Sperm from the cauda were counted using a hemocytometer and assessed for viability, chromatin damage, and morphological aberrations. Results demonstrate that mice exposed to 10%, 25% and 50% dilutions of e-waste demonstrate a dose-dependent increase (compared to control) in sperm chromatin damage (17, 34, 68%, respectively), suggesting DNA damage, and morphological abnormalities (11, 42, 70%, respectively) that included amorphous/missing heads, misfolded, doubled, or missing tails, and cytoplasmic droplets. As some toxic metal concentrations in the e-waste leachate mixture are higher than WHO standards (e.g., As, Cd, Hg), the same metals could contribute to the observed impacts on male reproduction. Implications of this study are significant, particularly for areas where e-waste dumpsite leachate can contaminate drinking water and soil. NYU GIPH & NYU NIEHS Center.

3249 Effects of Ethylene Glycolmonomethyl Ether on the Epigenetic Related Genes in the Testis of Male Rats

T. Fukushima[1], K. Taki[2], K. Masuno[1], C. Kondou[1], M. Ueno[1], T. Yoshida[2] and I. Horii[2]. [1]*Shionogi Co & Ltd, Toyonaka, Japan and* [2]*Showa University, Hatanodai, Japan.* Sponsor: Y. Tonomura.

The organic solvent ethylene glycol monomethyl ether (EGME) produces testicular lesion in rats and human. In the present study, to investigate epigenetic-related gene expression in EGME testicular lesion, mRNA and miRNA array screening and real-time RT-PCR analysis were conducted using testis collected from male rats given 2000 mg/kg EGME for one day. There was death of phacytene spermatocytes from 6hr after dosing and accompanied with Sertoli cell vacuolation after 24hr. From the mRNA expression analysis, DNA methyltransferase, dnmt3a, dnmt3b, and histone demethylase, jmjd3, were increased. On the other hand, histone deacetylase, hdac4, and histone methyltransferase, symd1, were decreased by EGME. Regarding miRNA, the expression of miR-449a and miR-141 was decreased, and the expression of miR-134 was increased. For the spermatogenesis, dnmt3a and dnmt3b contribute to progress or maintain of DNA methylation and associate with proliferation of spermatogonia or meiosis of spermatocyte. One of the target mRNAs of miR-141, jmjd3, was reported to work for proliferation of spermatogonia. Decreased expression of hdac4, the target gene of miR-134, suggests to histone hypermethylation. Additionally, decrease in symd1 is expected suppression of histone methylation. Decrease in miR-449a, which is known to be highly expressed in spermatocytes and spermatid, is supposed to be disorder of meiosis. Taken together, we considered EGME affected several epigenetics-related genes and maintaining DNA methylation or histone modification and finally, these things resulted in testicular lesion.

3250 Body Weights and Fertility of Male Rats Offspring after Prenatal Exposure to Bisphenol A

M. Elobeid and L. AlSadhan. *King Saud University, Riyadh, Saudi Arabia.* Sponsor: P. Goering.

Bisphenol A (BPA) can alter the hormone homeostasis and cause developmental disorders. The present study evaluated the effect of male rats' prenatal exposure to BPA on body and reproductive organs weights, fertility parameters and hormonal functions of offspring. Pregnant female rats were gavaged with BPA (1.2 and 2.4 µg/kg/day) plus a negative control during the prenatal period of six weeks. Adult first generation males (F1) were subjected to fertility assessment by mating with unexposed females to BPA. The reproductive functions of the subsequent second generation (F2) litters were investigated similarly. Hormonal levels and rat sperms qualities were investigated using an ELISA and the hemocytometer method, respectively. A significant increase was observed in number of corpora lutea, implantation sites, and post implantation loss in the females mated with the first generation offspring. Terminal body weights significantly increased as well as weights of the testes and the prostate glands across all generations. Contrarily, a significant decrease was observed in the epididymal weight, litter size, sperms count and motility. A significant decrease in follicle stimulating hormone levels across both generations F1 (51.25±1.93) and F2 (52.55±2.03) was observed. Luteinizing hormone levels showed a significant increase in the groups exposed to low doses of BPA in both generations F1 (46.53±0.15) and F2 (45.89±0.32). The plasma levels of the testosterone hormone were significantly decreased in the groups exposed to both doses of BPA of the F1 (13.06±0.28, 13.29±0.15, respectively) and the F2 (12.19±0.19, 12.74±0.23 respectively). And, only the groups exposed to low dose of BPA showed estradiol increase in both the F1 (350.73±11.51) and the F2 (259.89±2.44) generations. Prenatal exposure to environmentally relevant doses of BPA may lead to hormonal imbalances associated with body weights disruption and the male germ line leading to subsequent impairments in the offspring and their subsequent generations.

3251 Cell-Based High-Content Analysis (HCA) Reveals Differential Effects of BPA and Its Selected Analogues on Spermatogonia Stem Cells

S. Liang, L. Yin and X. Yu. *University of Georgia, Athens, GA.*

Bisphenol A (BPA), an endocrine-disrupting compound, is found to be a testicular toxicant in animal models. Bisphenol AF (BPAF), Bisphenol S (BPS) and Tetrabromobisphenol A (TBBPA), structurally similar to BPA, are introduced to the market as alternatives to BPA. However, there is limited toxicological data on the male reproductive systems of these compounds so far. In this study, we compared the time- and dose-dependent effects of BPA and its analogues on the cell viability and cell cycle alteration in spermatogonia stem cells. We observed dose-dependent decreases of cell viability using neutral red uptake assay at 24 and 48 h after treatments. The IC50 values at 48 h were 55.5, 58.5, 184.5 and 357.1 uM for TBBPA, BPAF, BPA and BPS, respectively. We established a high-throughput and high-content analysis (HCA) for the morphology and cell cycle analysis. The Hoechest 33342 staining was used to determine the nuclear size and nuclear morphology. 5-bromo-2´-deoxyuridine (BrdU) was used to label DNA synthesis for the active proliferating cells. Automated multi-channel images were acquired using ArrayScan VTI and quantified by HCS Studio 2. We found that BBAF and TBBPA significantly induced changes of nuclear shape at lower concentrations as compared to BPA and BPS, indicating a drastic condensation of chromatin. BBAF and TBBPA also significantly decreased the BrdU incorporation at non-toxicity doses at 72 h after treatments. In summary, our current study demonstrated the differential toxicity of BPA and its analogues on the spermatogonia cells. (supported by R21 OH 010473, ARDF and the UGA Startup Research funding.

3252 High Content Analysis (HCA) of Effects of Low-Dose Cadmium on DNA Damage and Cell Cycle in Spermatogonial Stem Cells

L. Yin, S. Liang and X. Yu. *University of Georgia, Athens, GA.*

Environmental and occupational exposures to cadmium (Cd) pose significant health risks to humans, including reproductive and developmental toxicity. Our previous studies have demonstrated that Cd at the concentration of 10 µM induced activation of stress signaling pathways, disruption ubiquitin-proteasome system and cell cycle alterations in primary rat testicular cell co-cultures. However, the testicular toxicity of Cd at environmentally relevant low doses has not been characterized. In this study, we established a high-throughput and high- content analysis (HCA) for cell cycle (Click-iT EdU and Phospho-Histone H3 Assay), DNA damage (γ-H2AX) and cytoskeletal structure (Phalloidin F-actin).

We applied this novel multiplex HCA analysis to examine the effects of Cd within a wide range of concentrations on the spermatogonial stem cells. We observed Cd induced a time and dose-dependent changes of cell cycle distribution, a significant decrease in S phase cells (EdU positive cells) and an increase of M phase cells (positive phospho-Histone H3 staining) in spermatogonial stem cells, even at a low dose of 0.50 µM. We found Cd induced dose-dependent increase of phosphorylation of γ-H2AX, a biomarker for DNA double-strand breaker. The increases of γ-H2AX were mostly observed in the S phase cells, but not in the M phase. Interestingly, these γ-H2AX positive stained cells were co-localized with the disruption of F-actin, suggesting the disruption of the actin cytoskeleton might be associated with DNA damage. Collectively, our current multiplex HCA analysis in the *in vitro* spermatogonia cells demonstrated that Cd at a low concentration could result in significant alteration of the cell cycle, cytoskeleton structure and DNA damage (supported by R21 OH 010473, ARDF and the UGA Startup Research funding).

PS 3253 Effects of Perfluorinated Compounds on Lipid Metabolism During *In Vitro* Spermatogenesis

D. Clarkson-Townsend[2], A. N. Steves[1], J. M. Bradner[2], W. M. Caudle[2] and C. A. Easley[1]. [1]*Cell Biology, Emory University School of Medicine, Atlanta, GA and* [2]*Environmental Health, Emory University Rollins School of Public Health, Atlanta, GA.*

Perfluorinated compounds (PFCs) are widespread, bioaccumulative persistent organic pollutants whose long half-lives present a chronic environmental exposure to the U.S. population and occupationally exposed workers. Previous epidemiological studies have found associations between PFC exposure and health outcomes related to lipid dysregulation, but these studies have not evaluated the effects of exposure on male fertility. Here using a novel, *in vitro* spermatogenesis human stem cell model, we tested the effects of chronic exposure to one particular PFC (perfluorooctane sulfonic acid (PFOS)) on germ cell viability, spermatogenic gene expression, and gene expression relating to lipid metabolism. Our results indicate that chronic PFOS exposure significantly altered spermatogenic and lipid metabolism gene expression. qPCR results showed differential expression of ACOX1, HCAR1, LIPE, PAX8, POMC, PON1, and SCYP3 following exposure to PFOS. Furthermore, exposure to PFOS significantly affected germ cell viability and appeared to specifically target spermatogonia viability as we observed a significant decrease in PLZF+ spermatogonia during PFOS exposure. These results suggest that prolonged, occupational-level PFOS exposure may negatively affect male fertility. We are currently assessing 3 other PFCs (perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS) and perfluorononanoic acid (PFNA)). Furthermore, we plan to evaluate whether occupational exposure to PFCs alters epigenetic landscapes in *in vitro*-derived spermatids to determine whether PFC exposure causes transgenerational effects.

PS 3254 Diabetes Upregulates Metastasis-Associated Protein-1 Signaling and Phosphorylation of Histone 2AX in Rat Testis

N. Kilarkaje. *Anatomy, Kuwait University, Safat, Kuwait.*

Diabetes induces reproductive impairments in both males and females. In males, diabetes causes the onset and progression of testicular dysfunction although the molecular mechanisms responsible for this effect are not clearly known. The present study investigated effects of diabetes on metastasis-associated protein-1 (MTA1) and phosphorylated-histone 2AX (p-H2AX), which are involved in DNA damage signaling and repair in rat testis. Adult male rats (13-16 week-old) were randomly divided into 4 groups (n=6); 2 groups served as controls and the rats in other two groups were given single ip injection each of Streptozotocin (55 mg/kg) to induce diabetes. Measurement of blood glucose levels throughout the study showed that the diabetic rats had severe hyperglycemia. The testes were sampled on 30d and on 90d following the confirmation of diabetes and the organs were processed for Western blotting and immunohistochemistry. Diabetes reduced weights of testes, epididymis, prostate, and seminal vesicles in a time independent manner. Diabetes increased MTA1 levels on 30d and 90d, but the increase was more on 30d than that on 90d and the increased protein expression was independent of seminiferous epithelial stages. All germ cell types and somatic cells expressed the protein in their nuclei. The p-H2AX level, on the other hand, increased in a time-and seminiferous epithelial stage-dependent manner. Mainly, spermatogonia and primary spermatocytes showed the phosphorylation of H2AX. In conclusion, these results indicate that diabetes up-regulates MTA1 signaling and phosphorylation of H2AX in testicular cells indicating DNA damage (Supported by Kuwait University grant # RM01/12 and SRUL02/13).

PS 3255 Increased Sertoli Cell Expression of Il-6 As A Mechanism for Mono- (2- Ethylhexyl) Phthalate Sensitivity in Pubertal Rats

A. R. Stermer, C. J. Murphy and J. H. Richburg. *Pharmacology and Toxicology, College of Pharmacy, University of Texas at Austin, Austin, TX.*

The testicular immune environment and spermatogenesis are regulated by cytokines that are produced by various testicular cell types. We recently reported that infiltration of macrophages (MΦ) into the testis correlates with the extent and timing of germ cell (GC) apoptosis after postnatal day (PND) 28 Fischer rats were treated with the Sertoli cell (SC) toxicant, mono-(2-ethylhexyl) phthalate (MEHP) (1.0 g/kg, p.o.). The MΦ within the testis are shown to be mostly ED2+ (anti-inflammatory) in control rats, however, after MEHP treatment *in vivo* a significant increase in ED1+ (pro-inflammatory) MΦ occurs at 12 and 24 h, signifying a shift in the normal testicular immune environment after MEHP exposure. To better understand this shift, the production of cytokines/ chemokines and their cellular source were evaluated through qPCR. IL-6 mRNA levels increased after exposure to MEHP (200 µM) in primary rat SC/ GC cultures at 3, 6, and 24 h and the SC line, ASC-17d, at 6, 12, 24 h. When Zileuton (0.5 µM), a 5-lipoxygenase (5-LOX) inhibitor, was added to ASC-17d cells in combination with MEHP, the observed increase in IL-6 mRNA was inhibited. Direct MEHP exposure to testicular MΦ and bone marrow derived macrophages (BMDMs) *in vitro* did not alter TNF-α nor IL-10, while MEHP-conditioned media from SCs significantly increased TNF-α at 24 h in BMDMs. Taken together, these results indicate that MEHP exposure increases SC IL-6 expression, which then may play a role in both exacerbating GC apoptosis and instigating macrophage infiltration, possibly through the induction of the 5-LOX/ IL-6 pathway.

PS 3256 Reproductive Assessment of Peginterferon Lambda-1a in Mice

L. Sivaraman[1], J.-J. Liaw[1], D. Bounous[1], K. Brannen[2] and M. E. McNerney[1]. [1]*Bristol-Myers Squibb Company, New Brunswick, NJ and* [2]*Charles River, Horsham, PA.*

BMS-914143 is PEGylated recombinant human interlukin-29/interferon lambda (IFN-λ1a). As part of the nonclinical safety evaluation, studies that investigated the effects of BMS-914143, administered subcutaneously, on fertility, embryofetal development (EFD) and pre-postnatal development (PPD), were conducted in Crl:CD1(ICR) mice at doses ranging from 10 to 500 µg/kg/day (µkd). In the fertility study, BMS-914143, at doses of 10, 50, or 500 µkd, had no effects on parental wellbeing or male reproductive parameters. However, in BMS-914143-treated female mice, at cesarean section on Gestation Day 13, at ≥ 50 µkd, there was a dose-related increase in postimplantation loss and at 500 µkd, corpora lutea were decreased and preimplantation loss was increased. In the EFD study, at doses of 20, 100, or 500 µkd, there were no BMS-914143-related maternal changes. Conversely, at cesarean section on GD 18, at ≥ 20 µkd, developmental toxicities included dose-related decreases in fetal body weights, increased incidences of fetal axial and appendicular malformations and skeletal variations. Additionally, at ≥ 100 µkd, preterm deliveries on GD 17 or 18 and fetal mortality (late resorptions and dead fetuses) were increased. In the PPD study, BMS-914143 was administered to female mice at 10 or 20 µkd from GD 6 to Lactation Day (LD) 20. To ensure sufficient number of pups for postnatal evaluations, an additional group of female mice (group 0/20 µkd) was administered BMS-914143 from GD 16 to LD 20. There were no adverse findings in dams. In F0 offspring, adverse decreases in pup weights at birth (all groups) that persisted to prepubertal period (at 0/20 µkd) or adulthood (at 10 and 20 µkd) were noted. Additional effects at 10 and 20 µkd were limited to perinatal mortality with associated decreases in live litter size on Postnatal Day 4. Overall, embryofetal and pup viability or growth is affected from implantation to adulthood when exposure to BMS-914143 is via the placenta or milk and these data are consistent with the reproductive effects of other interferons.

3257 Toxic Hazards Caused by Methanolic Extract of *Euphorbia peplus* in Male Rats

A. H. Abou Hadeed, G. G. Moustafa, A. T. Mohammed, F. H. Kandeel and A. M. Alkelech. *Department of Forensic Medicine and Toxicology, College of Veterinary Medicine, Zagazig University, Zagazig, Egypt*. Sponsor: A.-R. Kadry.

Euphorbia peplus has long been used as therapeutic remedy for the treatment of common skin lesions, actinic keratosis, and non-melanoma skin cancer. Despite its potential beneficial effects, increasing evidence suggesting that *Euphorbia peplus* may pose increased risk of human developmental or reproductive outcomes. However, the findings remain largely inconclusive. The purpose of the present study is therefore to investigate the toxic effects of *Euphorbia peplus* on male albino rats (fertility, DNA and hepatic tissue). In the study a total of 20 male albino rats dosed orally at 500 mg/kg BW *Euphorbia peplus*, obtained by methanolic extraction at room temperature, twice weekly for 65 days before sacrifice. The results of the histopathological examination showed that there was testicular degeneration, significant decrease in seminal vesicle weight, sperm concentrations, as well as sperm motility. There was also a significant increase in the percentage of sperm abnormalities as compared to controls. The extent of abnormal DNA fragmentation in both testicular and hepatic tissues has likewise increased in the *Euphorbia peplus*-treated rats. In summary, the current preliminary findings heighten the importance of weighting the risks and benefits involved in the use of *Euphorbia peplus* for therapeutic purpose. Further investigation is clearly warranted to fully understand the potential of *Euphorbia peplus* for disease treatment.

3258 Prenatal Exposure to Perfluorooctanesulfonic Acid (PFOS) Is Associated with Wheeze in Early Childhood

A. Impinen[1], U. C. Nygaard[1], K. C. Lødrup Carlsen[2,3], P. Mowinckel[2], K.-H. Carlsen[3] and B. Granum[1]. [1]*Department of Food, Water and Cosmetics, Norwegian Institute of Public Health, Oslo, Norway;* [2]*Department of Pediatrics, Oslo University Hospital, Oslo, Norway and* [3]*Institute of Clinical Medicine, University of Oslo, Oslo, Norway*. Sponsor: B. Granum.

Background: Prenatal exposure to PFASs (perfluorinated alkyl substances) is reported to be associated with immunosuppression in early childhood, but with contradictory findings related to other lung-related health outcomes. The aim of our study was to examine if prenatal exposure to PFASs is associated with lung function, asthma and wheeze in early childhood. Material and methods: Six PFASs were measured in cord blood available from 641 mothers in the Environment and Childhood Asthma birth cohort study (ECA). Health outcomes included lung function at birth by tidal breathing measures (flow-ratio tPTEF/tE) (n=435), and wheeze and asthma up to 10 years of age. Nonparametric comparisons were used to test significant differences of PFAS levels (ng/mL serum) in binary outcomes and logistic regression models to analyze associations between PFASs and health outcomes stratified by sex (54% boys) due to differences in disease prevalence. Results: Compared to girls, boys had more often asthma ever (34% vs. 20%) and wheezing ever (48% vs. 28%), with similar lung function at birth. In boys, lung function was positively correlated with PFOS (r=0.16; p=0.019), perfluoroundecanoic acid (PFUnDA) (r=0.13; p=0.043), and perfluorononanoic acid (PFNA) (r=0.16; p=0.018), whereas PFOS was associated with wheezing ever (OR 1.11; CI95% 1.00-1.24). No associations were found between PFASs and asthma. No significant associations in PFASs and measured health outcomes were found in girls. Conclusion: PFOS, PFUnDA and PFNA were significantly, but weakly, correlated to lung function at birth, whereas no associations between PFASs and wheeze were found. We have previously reported that reduced lung function at birth is associated with increased risk of asthma at 10 years of age. Our results thus indicate that prenatal exposure to PFASs do not affect lung function and asthma development. Prenatal exposure to PFOS was, however, associated with wheeze. Since wheeze may be induced by infections, our findings may be caused by immunosuppressive effects of PFOS.

3259 Comparative Reference Control Data for Embryo/Fetal Development Studies in Sprague-Dawley Rats (Crl CD® IGS BR vs. RjHan:SD) and New Zealand White Rabbits (Crl:KBL vs. INRA A 1077)

G. Chevalier, S. Bentz, F. Lesueur, M. Da Silva, R. Forster and F. Spezia. *Citoxlab, Evreux, France*.

Developmental toxicity studies are routinely performed at CiToxLAB France in Sprague-Dawley rats and New Zealand White rabbits, and the animals are supplied by two breeders per species. This presentation provides and compares background control data on the hysterectomy and fetal parameters of two strains per species. Data were collected from control animals of studies compliant with OECD test guideline No. 414 or ICH S5 design. In rat studies, the strain was either Crl CD® IGS BR from Charles River Laboratories, Italy, or RjHan:SD from Janvier LABS, France. The rabbit strains were Crl:KBL from Charles River Laboratories, France, or INRA A 1077 from Centre Lago, France. The comparison of pregnancy rate, mean pre- and post-implantation losses, mean number of live fetuses, mean fetal weight, and sex ratio did not highlight any obvious strain differences in any species. Regarding fetal examination parameters, fewer skeletal variations and external, visceral and skeletal malformations were obtained in our studies with the RjHan:SD rat strain than with the Crl CD® IGS BR rat strain. In rabbits, the incidence of litters with variations and malformations was evenly distributed over the two New Zealand White strains; Crl:KBL litters showed more external and skeletal variations while the INRA A 1077 strain had a higher litter incidence of visceral variations, but there were no marked differences in litter malformation incidence. These reference control data will be useful for the interpretation of on-going and future studies.

3260 Oral Embryofetal Toxicity Assessment of the Non-Narcotic Antitussive Benzonatate in Rats and Rabbits

S. Engel[2], V. Sharper[1] and S. Teo[3]. [1]*Charles River Laboratories, Horsham, PA;* [2]*Pfizer, Groton, CT and* [3]*Pfizer, Madison, NJ*.

Benzonatate TESSALON®) is a non-narcotic, peripherally acting, oral prescription antitussive for the symptomatic relief of cough. It is an ester typeanesthetic structurally related to procaine and is thought to inhibit cough by anesthetizing pulmonary stretch receptors which mediate the cough reflex. It is rapidly metabolized by body esterases to the major metabolite 4-(butylamino)benzoic acid (BBA). The embryo-fetal toxicity potential of Benzonatate (B) was determined in rats and rabbits. Doses of 0 (sterile water) 100, 200 and 250 mg/kg/day of B were orally gavaged to pregnant Crl:CD(SD) rats 25/group) from Gestation Days (GD) 7-17. Doses were based on a dose ranging study. Exposure was confirmed by toxicokinetic (TK) sampling of maternal blood samples on GDs 7 and 17 and fetal blood on GD 18. Benzonatate plasma levels were too low for quantifiable determination of TK parameters while BBA was detectable in maternal and fetal plasma. The 250 mg/kg dose had maternal free plasma BBA AUC (GD 17) that was 180X higher than that from a maximum daily human dose of 600 mg. There were no adverse effects of B on maternal parameters. There were also no effects on ovarian or uterine parameters or embryo-fetal survival and no gross external, soft tissue or skeletal fetal malformations or variations at any dose. The maternal and developmental no-observable-effect level (NOAEL) was therefore 250 mg/kg. Pregnant NZ White [Hra:(NZW)SPF] rabbits (20/group) were orally gavaged with 0 (sterile water), 500, 1000, 1250 mg/kg B from GD 7-19. Doses were based on a dose ranging study. Maternal TK blood was sampled on GDs 7 and 19 and fetal blood on GD 20. Both B and BBA were quantifiable in maternal blood but BBA levels were 28-300X higher than that of B. Neither were detected in fetal blood. The 1250 mg/kg dose had maternal free plasma BBA AUC (GD 19) that was 250X higher than that from a maximum daily human dose of 600 mg. The 1000 and 1250 mg/kg doses had adverse clinical observations, mortality (1 at 1250 mg/kg) and abortions (2 at 1000 mg/kg). There were reduced body weight gains/losses and feed consumption at all B doses. The maternal NOAEL of B was <500 mg/kg. There was no gross external, soft tissue or skeletal fetal malformations or variations. The developmental NOAEL was therefore 1250 mg/kg. In summary, the major B metabolite BBA was not teratogenic at maternal exposures that were significantly higher (180X for rat; 250X for rabbit) than that from a maximum daily human dose.

3261 *In Utero* Exposure to the Dietary Supplement Vinpocetine Is Associated with Rat and Rabbit Embryo-Fetal Toxicity

N. Catlin[2], P. Foster[2], E. Mylchreest[1], L. Miller[1], H. Cunny[2] and B. McIntyre[2]. *[1]Southern Research Institute, Birmingham, AL and [2]Program Operations Branch, National Toxicology Program, Durham, NC; [3]Toxicology Branch, National Toxicology Program, Durham, NC.*

Vinpocetine, a semi-synthetic derivative of the Vinca minor extract vincamine, inhibits phosphodiesterase type 1 and voltage-sensitive Na2+ channels, and display vasodilatory activity in cerebral tissue. Human exposure occurs through its use as dietary supplement for its purported nootropic and neuroprotective effects. Rat and rabbit prenatal toxicity studies were conducted to characterize potential maternal and embryo-fetal toxicity. Vinpocetine was administered via gavage to time-mated Hsd rats (n = 25) at dose levels of 0, 5, 20, and 60 mg/kg from gestation day (GD) 6-20, and time-mated NZW rabbits (n=8) at dose levels of 0, 25, 75, 150, and 300 mg/kg from GD 7-28. Pregnant rats administered vinpocetine exhibited dose-related decreases in body weight gain, as well as concomitant increases in vaginal discharge that were observed from GD 13-19. At necropsy, dams exhibited dose-dependent increases in post-implantation loss (3.3, 10.7, 11.1, and 83.1% at 0, 5, 20, and 60 mg/kg, respectively) that was associated with a higher frequency of early resorptions and fewer live fetuses at term. There were no effects on rat fetal weight or any external fetal findings. However, rat fetuses exposed to 0, 5, 20, and 60 mg/kg exhibited a dose-dependent increase in ventricular septum defects, with incidences of 0/293 (0/21), 3/239 (3/19), 8/261 (7/21), and 2/51 (2/8) of the fetuses (litters), respectively. In the pregnant rabbits, apparent maternal toxicity was minimal (sporadic decreases in food consumption). However, rabbits in the 300 mg/kg high dose group displayed post-implantation loss (20.4% vs 1.4% in controls), a decrease in the mean number of live fetuses, and fetal body weights that were 10% lower than controls. Taken together, these data indicate that vinpocetine administration to pregnant rats and rabbits results in embryo-fetal toxicity in both species, suggesting a potential hazard for pregnant women who may be taking vinpocetine.

3262 An Evaluation of the Effects of N91115 on Embryo-Fetal Development and Toxicokinetics of Pregnant Rabbits

D. Colagiovanni. *Product Development, Nivalis Therapeutics, Boulder, CO.*

N91115 is a clinical candidate for cystic fibrosis. N91115 is an S-nitrosoglutathione reductase inhibitor with activity that not only inhibits this enzyme, but, stabilizes CFTR at the cell membrane. Pregnant New Zealand White rabbits were evaluated following N91115 administration for effects on offspring. Maternal toxicity and plasma toxicokinetics (TK) in the pregnant does were measured. In addition to embryo-fetal skeletal and visceral assessments, tissue drug concentrations were assessed in the fetuses. Timed pregnant does were administered N91115 from gestation day (GD) 7-19. N91115 was given by oral gavage. Does were euthanized on GD 29 with a gross necropsy (c-section) and fetal assessments. Plasma TK were measured in the does on GD 7 and 19. After the final blood collection on GD 20, fetuses were collected and analyzed for total fetal tissue drug concentrations. N91115 oral doses were well absorbed and plasma exposure was linear with an increase in dose. A dose of 50 mg/kg/day was well tolerated by the does; higher doses of 150 and 300 mg/kg/day lead to maternal toxicity. Signs of maternal toxicity included decreased food consumption and one maternal death at 150 mg/kg/day, and decreased maternal body weight gain and food consumption, one maternal death, and early termination (due to fetal loss) in multiple does at 300 mg/kg/day. An increased rate of fetal resorption and post-implantation loss, as well as decreased fetal viability, was also noted in the high dose group. This dose (300 mg/kg/day) of N91115 corresponded to a maternal Cmax of 134 µg/mL AUClast and a measured fetal mean concentration of 0.076 µg/g of tissue. Overall fetal exposure was <1.0% of maternal exposure. External, visceral, and skeletal fetal examinations showed no significant findings at any dose level tested. The no-observed-adverse-effect-level dose of 50 mg/kg/day corresponded with a Cmax of 14.6 µg/mL. While maternal toxicity was noted in the study, overall results demonstrated N91115 did not affect the offspring.

3263 Prenatal Administration of Valproic Acid Alters Both the Purkinje Cell Dendrites and the Granule Cell Proliferation in Rat Developing Cerebellum

S. Yoshida[2], N. Hozumi[2], Y. Fueta[3], S. Ueno[3] and Y. Sekino[1]. *[1]National Institute of Health Sciences, Tokyo, Japan; [2]Toyohashi University of Technology, Toyohashi, Japan and [3]University of Occupational and Environmental Health, Kitakyushu, Japan.*
Sponsor: Y. Fueta.

Multiple environmental agents, including medical drugs, have been associated with an increased risk of autism. Valproate (VPA) is an antiepileptic drug known to be positively correlated with the occurrence of autism in the offspring of mothers exposed to the drug during pregnancy. In autism, some structural abnormalities in the cerebellum have been reported. Especially, a reduction in size and number of Purkinje cells are revealed in both the postmortem human studies and VPA-administrated adult animals. Now we investigated the effects of prenatal administration of VPA and observed their postnatal cerebellar development in young rat. VPA was once administrated to embryonic day 16 p.o., and the cerebellum in pups was observed from postnatal day 7 (P7) to P21. In normal cerebellar development, the soma of Purkinje cells form a single layer and elongate their dendrites with synapses during the first two weeks. In addition, ATP release induced by glutamate was drastically increased from P9 in response to synaptogenesis between Purkinje cells and granule cells. In VPA administrated rat, the elongation of Purkinje cell dendrites started earlier and reached all over the molecular layer even in P12. ATP release in VPA-administrated rat cerebellum revealed earlier and larger than it in normal rat cerebellum. In VPA-administrated rat cerebellum, furthermore, the lobules in the vermis developed earlier and formed excess gyrus in the anterior lobe, specifically in lobules V, VI and VII in P16. Granule cells proliferated even in the external germinal layer in P16. Both precocious development of Purkinje cells and elongated proliferation of granule cells are observed in the developing VPA-administrated cerebellum. Even in human, transient abnormal growth trend of the autistic cerebellum was reported. It was suggested that VPA-induced epigenetic effects would change the developmental progress in immature cerebellum and corrupt the cerebellar networks through adulthood.

3264 Prenatal Single Administration of Tributyltin Alters Developmental Transient of Spontaneous Behaviors and Basic Excitability in the Hippocampal Slices of Immature Rats

Y. Fueta[3], Y. Sekino[1], S. Yoshida[2] and S. Ueno[3]. *[1]National Institute of Health Sciences, Tokyo, Japan; [2]Toyohashi University of Technology, Toyohashi, Japan and [3]University of Occupational and Environmental Health, Kitakyushu, Japan.*

To evaluate the developmental neurotoxicity of chemicals, we have applied electrophysiological approach using brain slices of rat pups prenatally exposed to chemicals of interest. Valproic acid (VPA) is known as a developmental neurotoxicant because animal models of autism spectrum disorder has been established by prenatal exposure to VPA, and we have previously reported that a single VPA administration to pregnant rats hastens developmental transient of neuronal excitability and changes sensitivity to bicuculline methiodide, an antagonist of GABAA receptors, in the hippocampus of rat pups at two weeks of age. This result raises the possibility that the appearance of developmental neurotoxicity of VPA is predicted using immature rats by our *ex vivo* approach. In the present study, to test whether our method is applicable to other chemicals, tributyltin (TBT), a typical endocrine disrupter, was similarly administered to pregnant rats (20 mg/kg). We firstly measured the occurrence of the spontaneous behaviors of rat pups at PNDs 5 to 7. In the control pups, the behaviors decreased day by day, but not in the TBT-exposed pups, with showing significantly higher activity at PND 7 compared to the control pups. On the days from PNDs 13 to 18, following the behavioral test, field potentials were recorded from the CA1 area of hippocampal slices. Field potentials normally increase as growth during the period, but we found a significant decrease in the amplitude of population spike at PND16 in the TBT-exposed group compared to the control, which seems to be a retardation of developmental transient of excitability. Our results indicated that, unlike VPA, TBT delayed developmental transient of hippocampal excitability by prenatal single administration. Although neurobehavioral analysis in adolescent/adult period are needed to check the effect of this experimental dose of TBT, it is suggested that our electrophysiological approach using hippocampal slices obtained from juvenile rats may be useful to predict developmental neurotoxicity of chemicals occurring after adolescent period.

3265 NTE Protein and Encoding Gene in Neurodevelopment and Neurodevelopmental Toxicity

M. A. Sogorb[3], D. Pamies[1], J. Estevez[3], E. Del Rio[3], E. Fuster[3], C. Estevan[2] and E. Vilanova[3]. *[1]Center for Alternatives to Animal Testing (CAAT) at Johns Hopkins Bloomberg, Baltimore, MD; [2]European Chemical Agency, Biocide Unit, European Commission, Helsinki, Finland and [3]Institute of Bioengineering, University Miguel Hernandez of Elche, Elche, Spain.*

It was previously demonstrated that knock-out mice for the two alleles of the gene (Pnapla6) codifying for NTE (Organophosphorus Neuropathy Target Esterase) were not viable, while knock-out animals for a single allele of this gene were viable but with serious impairments in nervous system development. It was also demonstrated that the embryonic development was stopped probably due to impairments in vasculogenesis. Our laboratory demonstrated using D3 mouse embryonic stem cells that silencing of Pnpla6 gene (the gene codifying for NTE) caused serious impairments in embryo development, specifically through alterations in tubulogenesis, which altered pathways for the formation of respiratory, neural, and vascular tubes. Human embryonal carcinoma stem cells NTera2/D1 (hNT2) were also used to establish the role of NTE on neurodevelopment finding that the silencing of Pnpla6 reduced to 20% the number of neurons developed *in vitro* after 13 days of differentiation together with absence of different neuronal markers and marked reduction of electrical activity after 66 days of culture. Pnpla6 silencing also altered several developmental processes, mainly neurogenesis and epithelium tube morphogenesis. In this same cellular model the exposure to the NTE inhibitor mipafox did not cause significant alterations on the developmental pathways. It is also demonstrated that the inhibition of NTE with mipafox on D3 mouse embryonic stem cells under spontaneous differentiation does not alter the expression of the same genes affected by Pnpla6 silencing. In conclusions, all these findings support the initial observations regarding the lack of viability of Pnpla6 knocked-out mice addressing to NTE an important role in development probably not related to its esterase associated activity.

3266 A Microphysiological Approach to Elucidate Gene-Environment Interactions Involving Hedgehog Signaling and Orofacial Clefting

B. P. Johnson[2], D. M. Fink[2], D. J. Beebe[2] and R. J. Lipinski[2]. *[1]Biomedical Engineering, University of Wisconsin, Madison, WI and [2]Comparative Biosciences, University of Wisconsin, Madison, WI.*

Orofacial clefts (OFCs), including cleft lip and cleft palate are common and devastating birth defects affecting approximately 1 in 700 newborns worldwide. Human studies strongly suggest that OFC etiology involves the interaction of multiple, yet to be identified predisposing genetic mutations and environmental influences. We have shown that chemical antagonism of epithelial-mesenchymal Hedgehog signaling during acute "critical windows" in development results in reduced proliferation, tissue deficiency, and OFCs in the mouse. These findings definitively link a specific developmental pathway, which is sensitive to both genetic and chemical modulation to a human disease of poorly defined etiology. A tractable screening-based approach is needed to identify and functionally test Hedgehog pathway disrupting environmental factors, particularly in the context of genetic predisposition, but is not currently available. Here, we present a novel microphysiological culture model that recapitulates the molecular and cellular sequence of normal and abnormal lip/palate development. We show that embryonic facial growth processes can be biomimetically modeled *in vitro* by culturing an oral ectoderm monolayer over 3D embedded cranial neural crest mesenchymal cells. These 3D tissue processes are supported by a natural extracellular matrix and engineered to mimic the spatial relationships, normal outgrowth, and appropriate perfusion of the facial processes. Cultures are evaluated for endpoints along the adverse outcome pathway including antagonism of Gli activation, proliferation and outgrowth. Future studies will test responsiveness of the model to genetic perturbation of known susceptibility genes. The ultimate goal is to develop preventative strategies through identification of at risk patient populations and culpable environmental agents bringing widespread translational potential for improving human health.

3267 Mechanism Underlying Offspring Undergrowth Produced by Maternal Exposure to Dioxin: A Study Focusing on Growth Hormone During the Fetal Period

Y. Hattori[1,2], T. Takeda[2], Y. Ishii[2] and H. Yamada[2]. *[1]Japan Society for the Promotion and Science, Research Fellowship for Doctoral Course Students, Tokyo, Japan and [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan.*

Maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at lower doses causes many developmental disorders such as growth retardation and sexual immaturity in offspring. Our previous studies have demonstrated that TCDD attenuates the pituitary expression of luteinizing hormone (LH) in fetuses, resulting in the impairment of sexual behavior after reaching maturity. Furthermore, we have recently revealed that TCDD produces the lowered expression of growth hormone (GH) in the fetal pituitary due, at least in part, to an attenuation in the circulating level of glucocorticoid, a proliferator of GH-producing cells, both in mothers and their fetuses. However, the mechanism underlying such attenuation and its relevance to developmental disorders remain obscure. To address these issues, we initially investigated the effect of TCDD on the number of GH- and LH-producing cells in the fetal pituitary. Single oral administration of TCDD (1 μg/kg) to pregnant rats at gestational day (GD) 15 reduced the number of GH-producing cells in the fetal pituitary, whereas the same was not true for LH-producing cells. In accordance with this, TCDD attenuated the serum concentration of insulin-like growth factor-1 (IGF-1), one of the GH-regulated factors, as well as GH in fetuses. The direct supplementation of GH to the TCDD-exposed fetuses restored not only the reduced level of IGF-1 in the fetal liver at GD20 but also a TCDD-induced reduction in body weight and length in newborn pups. In addition, the damage by TCDD to learning memory, formation of bone and lipid metabolism in offspring after reaching maturity was also recovered or tended to be recovered by supplying GH during the fetal stage. These results suggest that TCDD attenuates the fetal production of GH by decreasing the number of GH-producing cells, resulting in the retardation of offspring growth.

3268 The TCDD-AHR Axis Targets MAP3K1 Signaling in Embryonic Eyelid Development

M. Mongan, J. Wang, Q. Meng, A. Puga and Y. Xia. *Environmental Health, University of Cincinnati, Cincinnati, OH.*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a prototype of ubiquitous organochlorinated pollutants capable of exerting toxicity on diverse developmental processes through mechanisms still poorly understood. Eyelid development requires fusion of upper and lower eyelid epithelia, occurring between gestational day (GD) 15.5 and GD16.5 in mouse fetuses. This process depends on a genetic network that regulates the MAP3K1-Jun N-terminal kinase (JNK) signaling cascade. Using mouse eyelid development as a model, we investigated the genetic and signaling mechanisms through which *in utero* TCDD exposure disturbs embryonic programs. Pregnant dams were treated with different doses of TCDD by oral gavage on different GDs and the eyelid of the pre-natal fetuses was examined. We found that although TCDD exposure did not affect eyelid closure in wild type fetuses, it blocked closure in Map3k1 heterozygous and Jnk1 homozygous mutant fetuses. Blocking eyelid closure corresponded to significant reduction of JNK activity in eyelid epithelial cells. Interestingly, alone neither TCDD nor the genetic conditions had an adverse effect on eyelid closure, suggesting that *in utero* TCDD exposure has to cooperate with specific genetic lesions to inactivate JNK and cause defective eyelid development. Most biological effects of TCDD are mediated by aryl hydrocarbon receptor (AHR), a ligand-activated basic helix-loop-helix-Per-Arnt-Sim transcription factor. Through a genetic approach, we showed that AHR ablation, in either whole body or ocular surface epithelia, abolished the TCDD effects on the eyelid of Map3k1 hemizygous fetuses. Taken together, our data show that the TCDD-AHR axis synergizes with genetic lesions, i.e. Map3k1 hemizygous and Jnk1 homozygous mutants, to inactivate JNK signaling and disturb eyelid development. The results support the notion that pre-existing genetic conditions can sensitize an individual to the developmental toxicity of TCDD.

3269 Neonatal and Juvenile Eyes Development in Sprague-Dawley Rats: A Histomorphological Study

R. Forster[1], V. Vrolyk[3], A. Apreutese[2], C. Gordon[2], A. Graham[2], B. Palate[1] and M. Benoît-Biancomano[3]. *[1]CiToxLAB France, Evreux cedex, France; [2]CiToxLAB North America, Laval, QC, Canada and [3]Faculté de Médecine Vétérinaire, Université de Montréal, Sainte Hyacinthe, QC, Canada.*

This study aimed at evaluating the histomorphological developmental features of the eye in Sprague-Dawley rats, with focus on the postnatal and juvenile periods. Tissues were collected from 51 rats, from both sexes, aged 1 to 30 days old. As in other altricial species, the eyes of newborn rats are underdeveloped and the eyelids remain closed for approximately two weeks after birth. During the postnatal development, dramatic changes occurred within the retina, when programmed cell death and cell differentiation coexisted. A complete separation of the inner nuclear layer from outer nuclear layer was first observed close to the end of the first week of life, while the nerve fiber, ganglion cell and inner plexiform layers almost reached maturity concurrently with the opening of the eyes, around the end of the second week. At birth, apoptotic bodies were frequently seen within the undifferentiated nuclear layer and peaked at post-natal day (PND) 8 within the inner nuclear layer. In newborn animals, mitoses formed a contiguous band of proliferating cells within the undifferentiated nuclear layer, adjacent to the photoreceptors layer. Perinatally, the corneal stroma and sclera exhibited high cellularity and became thinner and less cellular with maturity. The corneal epithelium consisted of a single layer of non-stratified columnar cells during first ten PNDs, and transformed into 4-5 cell layers of stratified squamous epithelium at about PND 14. The lens continuously developed by addition of new lens fibers from the lens epithelium. Between PND8-PND17, a band-like region of apoptotic bodies was seen centrally within the zone of nucleated lens fibers. We conclude that, during the first month of life, the rat's eyes undergo a rapid phase of growth and almost reach adult maturity at PND30. These observations will serve as historical database, useful in pediatric ocular drug development.

3270 Neonatal Activation of the Xenobiotic-Sensor PXR Results in Acute and Permanent Down-Regulation of PPARα-Signaling in Mouse Liver

C. Y. Li, T. K. Bammler and J. Y. Cui. *Department of Environmental & Occupational Health Sciences, University of Washington, Seattle, WA.*

The pregnane X receptor (PXR) is a critical xenobiotic-sensing nuclear receptor that can be activated by many drugs and environmental toxicants. Recently, increasing safety concerns have emerged regarding the developmental exposure to xenobiotics and the potential long-lasting adverse effects beyond childhood. The goal of this study was to test our hypothesis that neonatal exposure to PXR-activators not only produces acute but also permanent changes in the expression of genes involved in xenobiotic and/or intermediary metabolism. The wild-type (C57BL/6J) mice were administered a single dose of the prototypical PXR-ligand PCN (75mg/kg, i.p.) or vehicle (corn oil, i.p.) at 3-day neonatal age. Livers were collected 24h postdose or at 60-day adult age, and the gene expression was determined using the Affymetrix Mouse Transcriptome Array 1.0. In neonatal liver, PCN up-regulated 464 and down-regulated 449 genes. In addition to the expected up-regulation of many well characterized PXR-targeted drug-processing genes, neonatal exposure to PCN also up-regulated the prototypical aryl hydrocarbon receptor target gene cytochrome P450 (Cyp) 1a2 in neonatal liver, as well as the constitutive androstane receptor target gene Cyp2b13 in adult liver. Pathway analysis demonstrated that PCN up-regulated the expression of genes involved in cell cycle, amino acid metabolism, and purine metabolism in neonatal liver. Interestingly, in newborns, PCN down-regulated multiple Cyp4a members, which are prototypical-target genes of the peroxisome proliferator-activated receptor alpha (PPARα), which is a critical nuclear receptor for lipid metabolism, and this down-regulation persisted to adulthood. RT-qPCR, western blotting and P450-Glo activity in wild-type and PXR-null mouse livers confirmed that both the acute and the permanent down-regulation of the Cyp4a genes were PXR-dependent. In conclusion, our study is the first to demonstrate that neonatal exposure to PXR activators leads to both acute and persistent changes in the hepatic expression of genes involved in drug and lipid metabolism. Most notably, there appears to be a neonatal PXR-mediated persistent decrease in PPARα-signaling in adult liver, suggesting that it is important to understand the connections between neonatal exposure to PXR-activators and the adult onset of certain intermediary metabolism-related liver diseases.

3271 Potential Modes of Action for Perfluorooctanoic Acid (PFOA)-Induced Hepatocellular Hypertrophy in Mice

E. Quist, V. Chappell, A. Filgo, Y. Wang, G. Kissling and S. Fenton. *NIEHS/NTP, Research Triangle Park, NC.*

Perfluorooctanoic acid (PFOA), a widely used industrial surfactant and persistent environmental toxicant, is thought to induce hepatocellular tumors via PPAR-α activation. Pregnant CD-1, 129/Sv and PPAR-α KO mice were orally gavaged with low doses of PFOA (0, 0.01, 0.1, 0.3, 0.6, and 1 mg/kg) from gestation days 0 through 17. Livers were collected on post-natal day (PND) 91 and routinely processed for histological evaluation, transmission electron microscopy (TEM) and DNA microarray analysis. In PFOA-exposed CD-1 and 129/Sv offspring, pronounced hepatocellular hypertrophy was histologically evident at PND 91. Liver TEM at PND 91 also revealed PFOA-induced cellular damage and mitochondrial proliferation with altered morphologies suggestive of increased or uncontrolled fission and fusion reactions; significant peroxisome proliferation was not observed. Based on these findings, we conclude that prenatal exposures to low doses of PFOA induce hepatocellular hypertrophy in mice due to mitochondrial proliferation, not peroxisome proliferation. We further suggest that the induced mitochondrial changes in these prenatally exposed mice represent altered mitochondrial function that is driving the hypertrophy response, and, when prolonged, these alterations may lead to tumor development later in life. Ongoing microarray analyses of hepatic RNA isolated from affected mice, indicate that these prenatal PFOA exposures induce altered genomic expression of key regulators involved in lipid metabolism, cellular proliferation pathways and mitochondrial function. The PFOA doses evaluated in this study overlap with reported circulating serum PFOA levels in the general human population making these findings especially relevant.

3272 RNA-Sequencing Reveals Differential Expression and Enrichment of Human Placental Xenobiotic Transporters Across Gestation

A. Estevez[2,4], X. Wen[4], K. Bircsak[4], B. Wang[3], J. Landis[1], X. Tao[1], N. Treff[3], T. Rosen[3] and L. M. Aleksunes[4]. *[1]Foundation for Embryonic Competence, Basking Ridge, NJ; [2]University of Puerto Rico-Rio Piedras, San Juan, PR; [3]Department of Obstetrics, Gynecology and Reproductive Sciences, Rutgers University, New Brunswick, NJ and [4]Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

The maternal-fetal disposition of drugs, environmental contaminants, and toxicants is regulated in part by transporters expressed on placental trophoblasts. Uptake transporters including the solute carriers (SLC) and efflux proteins such as the ATP-binding cassette (ABC) transporters are expressed on the apical (maternal-facing) and basolateral (fetal-facing) portions of the plasma membrane and control the transplacental transfer of chemicals. The purpose of the present study was to assess temporal changes in the expression and enrichment of xenobiotic SLC and ABC transporters in cultured primary human trophoblasts from midterm (17–21 weeks, n=4) and full term (38-40 weeks, n=4) placentas from uncomplicated pregnancies using RNA-Sequencing and immunohistochemistry. At both times, the absolute expression of transporter mRNAs revealed greater enrichment of SLC transporters compared to ABC genes. The most highly expressed transporters included SLCO (OATP) 2B1, 2A1, 3A1, 4A1, SLC22A1 (OCT1), SLC22A3 (OCT3), 22A4 (OCTN1), 22A5 (OCTN2), SLC 22A11 (OAT4), SLC (ENT) 29A1, 29A3 and ABCC (MRP) 1, ABCC3, ABCC5, ABCG2 (BCRP). Transporter isoforms that were expressed to a greater extent at midterm included SLCO2B1 (20-fold), SLC22A5 (3-fold), and SLC29A1 (3-fold), A3 (2-fold). With advancing gestation, increases in the mRNA of SLCO3A1 (2.5-fold), 4A1 (7-fold), SLC22A1 (16-fold), and SLC22A3 (17-fold) were observed. Immunohistochemical staining is underway to confirm gestation-dependent expression of transporters. These findings reveal the absolute levels of transporter mRNAs in cultured primary human trophoblasts and highlight changes in expression across gestation that may alter the maternal-fetal disposition of potentially harmful chemicals. Supported by the SOT Intern Program and R25ES020721.

3273 Exposure to Butyl Paraben During Gestation and Lactation in Harlan Sprague Dawley Rats via Dosed Feed

G. K. Roberts[1], S. Waidyanatha[1], B. L. Fletcher[2], M. A. Silinski[2], T. R. Fennell[2], H. C. Cunny[1], V. Godfrey Robinson[1] and C. R. Blystone[1]. *[1]Division of the National Toxicology Program, Research Triangle Park, NC and [2]RTI International, Research Triangle Park, NC.*

Butyl paraben (BPB) is an antimicrobial used in a variety of consumer products. Due to widespread human exposure and reported estrogenic activity, the National Toxicology Program conducted a reproductive and developmental toxicity study in rats via dietary exposure. To evaluate exposure during critical periods of development, a separate study, presented here, was conducted to determine BPB levels in the fetus, amniotic fluid, and plasma. Time-mated female Harlan Sprague Dawley rats were administered 0, 1500, 5000 or 15000ppm BPB via NIH-07 feed, ad libitum, from gestation day (GD) 6 to postnatal day (PND) 28. Samples were collected on GD18 and PNDs 4, 10, 14, 21 and 28 and analyzed for free (unconjugated) and total (unconjugated and conjugated) BPB using liquid chromatography-tandem mass spectrometry. Free BPB (<5000ppm) and total BPB (1500ppm) levels were below the limit of quantitation in amniotic fluid and fetus, suggesting limited placental transfer. Total BPB in PND4 pup plasma was less than 5% of dam plasma for all doses, suggesting low lactational transfer. However, poor conjugation in pups resulted in higher plasma levels of free BPB compared with dams for nearly all time points and groups; for example, at PND10 in the 5000ppm group, total BPB was ~35 times higher in the plasma from dams versus pups, while free BPB was ~2 times higher in pup plasma. Pup conjugation of BPB was age and dose-dependent, not reaching the percent conjugation observed in the dams (>99%) until PND28. The ability of the pups to form oxidative metabolites was evaluated by analyzing butyl 3,4-dihydroxybenzoate (3-OH BPB). The ratio of total 3-OH BPB to total BPB at PND21 and 28 ranged from ~15-23% in pups and ~20-35% in dams. These data illustrate low placental and lactational transfer of dietary BPB and that poor conjugation in pups results in higher exposure to free BPB compared to dams.

3274 EU Pediatric Investigation Plans with Juvenile Animal Studies: 2007–2014

M. E. Hurtt and S. M. Engel. *Drug Safety Research and Development, Pfizer Inc, Groton, CT.*

The Pediatric Regulation came into effect in 2007 in the European Union (EU). The Regulation was designed to better protect the health of children in the EU. The main component was the need to submit a Pediatric Investigation Plan (PIP), which is a development plan for a drug's use in children. The submitted PIP must address clinical as well as non-clinical studies. The concern since the Regulation came into effect has been raised about the number of juvenile animal studies (JAS) being conducted in the EU and the number of species required for those studies. The question of whether there has been an increase in the number of JAS requested by European Medicines Agency (EMA) and the question of single or multiple species studies are the focus of this abstract. All source data on PIPs were obtained from the EMA pediatrics website and evaluated by year as obtained for 2007-2014. There were 156 PIPs with JAS from 2007 through 2014. The analysis of the data originally showed the percent of PIPs with JAS was 0%, 23%, 26%, 7%, 19%, 17%, 25% and 33% for 2007 through 2014 respectively. The data suggested an increase in the percentage of PIPs with JAS in 2014 compared with previous years. For those PIPs with modifications, the EMA assigns a new date to the newly agreed modification and the original PIP no longer appears as the date of record. Therefore, the data were also adjusted to the date of the original PIP agreement rather than the newly assigned date of the PIP modification. A thorough examination of the adjusted PIP data from December 2007 through 2014 support that the percent of PIPs with JAS is not increasing and was 0%, 22%, 31%, 13%, 28%, 29%, 14% and 26% for 2007 through 2014 respectively. The data also supported that 94% of the JAS were proposed with a single species and that species was predominately the rat (80%) and that multi-species JAS for an agreed PIP were infrequent.

3275 Juvenile Development: When Normal Looks Abnormal; Part I. Nervous, Endocrine, and Reproductive Systems

A. K. Remick[2], C. A. Picut[2], D. L. Brown[2], B. E. Walling[1] and G. A. Parker[2]. *[1]Pathology, WIL Research, Ashland, OH and [2]Pathology, WIL Research, Hillsborough, NC.*

Dynamic tissue changes transpire during the postnatal period in rats as most tissues are not fully developed at birth. Pathologists and toxicologists should be aware of the range of changes that occur such that, at a minimum, changes that are normal in postnatal development will not be diagnosed as abnormal in toxicity studies. To investigate the range of normal developmental changes, tissues from male and female Sprague Dawley rats on postnatal days 1 through 42 were collected, processed, paraffin-embedded, stained with hematoxylin and eosin, and examined microscopically. Histologic changes in tissues of the nervous, endocrine, and reproductive systems were described and changes which may be mistakenly diagnosed as abnormal were selected and presented. Selected changes included neuronal pruning and apoptosis in the brain; cell population shift from basophil to acidophil predominance in the pituitary gland; emergence of sexual dimorphism in the thyroid gland; apoptosis in the testis; and atypical follicles and follicular atresia in the ovaries. Familiarity with the striking tissue changes occurring in postnatal rats will prevent inaccurate pathologic interpretations in toxicity studies. In addition, this knowledge will enhance the expertise of pathologists and toxicologists in juvenile toxicologic pathology and ensure quality in safety assessment studies for pediatric populations.

3276 Juvenile Development: When Normal Looks Abnormal; Part II. Respiratory, Digestive, and Excretory Systems

C. A. Picut[2], A. K. Remick[2], D. L. Brown[2], G. D. Coleman[1], M. A. Greeley[1], M. E. Mattix[1], J. T. Pike[2], L. Staska[2], B. E. Walling[1] and G. A. Parker[2]. *[1]Pathology, WIL Research, Ashland, OH and [2]Pathology, WIL Research, Hillsborough, NC.*

Dynamic tissue changes transpire during the postnatal period in rats as most tissues are not fully developed at birth. Pathologists and toxicologists should be aware of the range of changes that occur such that, at a minimum, changes that are normal in postnatal development will not be diagnosed as abnormal in toxicity studies. To investigate the range of normal developmental changes, tissues from male and female Sprague Dawley rats on postnatal days 1 through 42 were collected, processed, paraffin-embedded, stained with hematoxylin and eosin, and examined microscopically. Histologic changes in tissues of the respiratory, digestive, and excretory systems were described and changes which may be mistakenly diagnosed as abnormal were selected and presented. Selected changes included a double capillary network in the saccular stage of lung development; transition from saccular stage to alveolarization in the lung; vacuolation and extramedullary hematopoiesis in the liver; lipid and protein absorption vacuoles in the intestine; hyperplastic appearance of the gastrointestinal tract; and proliferation, apoptosis, and desquamation of the urinary bladder. Familiarity with the striking tissue changes occurring in postnatal rats will prevent inaccurate pathologic interpretations in toxicity studies. In addition, this knowledge will enhance the expertise of pathologists and toxicologists in juvenile toxicologic pathology and ensure quality in safety assessment studies for pediatric populations.

3277 NHP Infant ECG and Echocardiography

M. A. Bailey[2], S. Kosaka[2], M. Dabadi[2], L. Hagen[2], T. Massey[2], A. Walker[2], T. Beck[2] and R. Nagata[1]. *[1]Shin Nippon Biomedical Laboratories, Ltd., Tokyo, Japan and [2]Scientific Services, SNBL USA, Everett, WA.* Sponsor: T. Beck.

In human development there is natural stages from fetus to the neonate, infant, child and finally to adult. The development results in major changes in system functions, size and shape. Significant changes occur with regard to the function, size and structure of the cardiovascular system. These known changes in cardiac function are well documented as a human child grows. However, there is no research or background information published on the NHP infant. Echocardiography and ECG data were performed on NHP infants during early development. Echocardiography was collected 1-3 times at 10-27 days old, 68-69 days old and 279 days to 1 year old, along with ECG for 5 infants. Echocardiography was used to estimate right and left ventricle cardiac output volume. ECG was collected from 2 -60 day old and around 1 year old from 18 infant under sedation (52 ECGs) and awake (37 ECGs). R-axis, Heart rate, PR interval, QT interval and Bazett's corrected QT interval

(QTcb) were analyzed, and ECG data was compared with adult NHP (2-10 years old). The heart rate in NHP infants was observed to be higher than in adult. Although QTcb in the infants were variable, no significant difference was observed between infants and adults. Right Axis Deviation (RAD) was observed more often in infants than seen in adults. This RAD corresponds to the changes seen in echocardiography. RAD gradually decreased with a shift to the left as the infant grows. However, more variation was observed in infants than in adults. As seen in human heart development, it was also observed in NHP that left ventricle growth and corresponding cardiac /ECG change.

PS 3278 Fetal Cortisol Exposure: Maternal Influences and Developmental Trends in a Nonhuman Primate Model

K. S. Grant[3], J. Worlein[3], J. Meyer[2], M. Novak[2], K. Rosenberg[2], C. Kenney[3], R. Kroeker[3] and T. Burbacher[3]. [1]*Environmental and Occupational Health Sciences, University of Washington, Seattle, WA;* [2]*Psychology, University of Massachusetts, Amherst, MA and* [3]*Washington National Primate Research Center, University of Washington, Seattle, WA.* Sponsor: E. Faustman.

Chronic prenatal stress has been associated with fetal exposure to elevated levels of maternal cortisol. The adaptive changes made by the fetus in response to elevated maternal cortisol have been associated with permanent changes in neuroendocrine biophysiology and susceptibility to disease in adulthood. To explore normative cortisol physiology during pregnancy and infancy in a closely related species, hair samples were obtained from a sample of 33 healthy Macaca nemestrina mother-infant dyads not subjected to chronic stress. Samples were collected from adult females during a pregnancy-detection ultrasound and immediately following labor and delivery. Infants were nursery-reared and hair samples were collected at birth, 20 days, 60 days and every two months thereafter until one year of age. Hair cortisol concentrations (HCCs) were determined using an enzyme immunoassay in washed and ground hair samples. Maternal HCCs at pregnancy detection ($\dot{x}$=114.6 ±26 pg/mg) were highly predictive of maternal HCCs at delivery ($\dot{x}$=150.8±30 pg/mg). Infant birth HCCs ($\dot{x}$=975±170 pg/mg) were significantly higher than maternal HCCs at delivery, suggesting a marked degree of fetal cortisol exposure over gestation. While maternal HCCs were not correlated with newborn cortisol levels, the increase in maternal HCCs across pregnancy was related to infant hair cortisol at birth ($p \leq .02$). Infant HCCs showed a precipitous 68% decrease over the first three postnatal weeks. Higher HCCs at birth were strongly associated with larger decreases in infant HCCs during the first year of life. Increased concentrations of maternal cortisol associated with chronic stress during pregnancy should be considered within the category of relevant environmental exposures that can have enduring effects on child health and development. This research was funded by NIH grants P51OD010425 and R24OD011180.

PS 3279 Role of Ethanol in Enhancing Fluconazole-Related Teratogenic Effects on *In Vitro* Post-implantation Rat Embryo Development

F. Di Renzo[2], F. Metruccio[3], M. Battistoni[2], A. Moretto[2] and E. Menegola[2]. [1]*Biomedical and Clinical Sciences, University of Milano, Milano, Italy;* [2]*Biosciences, University of Milano, Milano, Italy and* [3]*International Centre for Pestides (ICPS), Luigi Sacco Hospital, Milano, Italy.*

Fluconazole (FLUCO) is a first generation azole-derivative antifungal drug. It is indicated for clinical use being water soluble and well absorbed after oral administration. FLUCO is generally well tolerated (probably the least toxic of all the antifungal drugs). The FDA pregnancy category for high dose fluconazole indications has been recently changed from category C to category D, while the pregnancy category for a single, low dose of fluconazole is category C. Ethanol (Eth) is a commonly consumed in alcoholic beverages also during pregnancy even if it is known to be a teratogen and its consumption during pregnancy can produce a wide range of adverse effects in the developing conceptus. The aim of this work is to evaluate the effects of the co-exposure of Eth and FLUCO on rat embryos *in vitro* development. For this purpose, E9.5 rat embryos were exposed *in vitro* to Eth (17-42.5-85-127 mM) or FLUCO (62.5-125-250-500 µM) alone or were co-exposed to the NOAEL of Eth (17 mM) and to the different concentrations of FLUCO or were co-exposed to the NOAEL of FLUCO (62.5µM) and to the different concentrations of Eth. At the end of the culture period (48h) embryos were morphologically examined in order to evaluate growth parameters and abnormalities. Based on bench mark dose (BMD) and relative potency factor (RPF) approaches, the outcome of mixtures were modelled and the predicted effects, derived under the dose-additivity hypothesis, compared with the experimentally obtained results. Concentration-related effects were observed after the exposure to Eth (42.5-127 mM) or FLUCO (125-500 µM). The observed abnormalities were specifically at the level of the branchial apparatus. The co-exposure to Eth and FLUCO at their NOAELs resulted effective, inducing almost 40% branchial arch abnormalities. Also, a significant enhancement of teratogenic effects were observed in the other groups co-exposed to FLUCO and Eth in comparison to the single exposure. Results from BMD approach suggest that there are no significant deviations from concentration-additivity. These findings might be relevant for the risk assessment of combined exposures. Supported by a grant Horizon 2020 SFS-12-2014 EUROMIX.

PS 3280 Effects of a Binary Mixture of Azole Fungicides on Post-Implantation Rat Embryo Developed *In Vitro*

F. Metruccio[3], F. Di Renzo[2], M. Battistoni[2], A. Moretto[2] and E. Menegola[2]. [1]*Biomedical and Clinical Sciences, University of Milano, Milano, Italy;* [2]*Biosciences, University of Milano, Milano, Italy and* [3]*International Centre for Pestides (ICPS), Luigi Sacco Hospital, Milano, Italy.*

Literature data indicate certain azole fungicides as potentially teratogenic, inducing craniofacial defects in different animal models. The *in vitro* exposure of rodent postimplantation embryos induces specific branchial arch (BA, the facial precursors) abnormalities. Recently, in analogy to their antifungal activity and to the mechanism proposed for their side effects (inhibition of CYP enzymes), the inhibition of embryonic CYP26 has been proposed as the common teratogenic mechanism. The aim of the present work was to evaluate *in vitro* the teratogenicity of the mixtures of two agrochemical azole fungicides: triadimefon (FON) and flusilazole (FLUSI). E9.5 rat embryos were cultured for 48h in presence of FON, FLUSI or mixtures of FON+FLUSI at increasing concentrations in order to delineate concentration-response curves for single compounds and for mixtures. At the end of the culture period, embryos were morphologically examined. Based on bench mark dose (BMD) and relative potency factor (RPF) approaches, the outcome of mixtures were modelled and the predicted effects, derived under the dose-additivity hypothesis, compared with the experimentally obtained results. Abnormalities were recorded only at the level of BAs. A clear concentration-response was observed both for the single fungicides and for mixtures. An additive effect was detected, confirming the hypothesis that FON and FLUSI act as teratogens via a common mode of action. These results focus attention on the possible additive effects of the multiple exposure to azole fungicides for mammalian developing embryos and therefore suggest that azoles should be grouped together for risk assessment. Supported by a grant Horizon 2020 SFS-12-2014 EUROMIX.

PS 3281 Abstract Withdrawn by Author.

PS 3282 Using Zebrafish to Assess Cannabinoid Developmental Toxicity

D. R. Carty, C. E. Thornton, S. J. Cutler, M. A. ElSohly, I. A. Khan and K. L. Willett. *Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS.*

While cannabidiol (CBD) and cannabidiol oil (CBO) show anecdotal efficacy in reducing seizure frequency in the treatment of pharmaco-resistant epilepsy, little is known concerning the potential adverse side-effects of CBD, CBO, and other structural analogues on child physiology, brain development, and subsequent adult disease. The goal of this project is to compare the relative molecular, morphological and behavioral phenotypes that result after developmental exposure to CBD, CBO, Δ9-tetrahydrocannabinol (THC), and other purified and synthesized cannabinoid analogues. Our study design, using an adverse outcome pathway paradigm, will allow us to compare molecular pathways and phenotypic outcomes of cannabinoids. We measure mortality at 24 and 96 hour post fertilization (hpf), touch response suppression, yolk sac and pericardial edemas, axis mutations, fin deformities, and gene expression of 15 mRNAs critical in morpho-/neurogensis. The LC50 for WIN 55,212-2 (synthetic cannabinoid) was 1.2 mg/L ± 0.19 (0.0022 mM). Developmental toxicities included decreased touch response (IC50 = 0.5 µM) and increased pericardial edemas. Ultimately, using this high-throughput developmental model, we hope to identify a relatively non-toxic cannabinoid with anti-seizure activity. Our comparative approach is highly relevant due to the known adverse effects of THC

exposure on humans including brain development deficits and social disorders, while the developmental toxicity of CBD, CBO and other analogues is unknown. Supported by Grant Number P20GM104932 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH).

3283 Estradiol Co-Exposure Rescues Certain Benzo[a]pyrene- and Cyp19a1b Knockdown-Mediated Developmental Phenotypes in Zebrafish

K. Alharthy, C. Thornton, J. Corrales and K. L. Willett. *BioMolecular Sciences, University of Mississippi, University, MS.*

Benzo[a]pyrene (BaP), a known developmental toxicant, inhibits aromatase (CYP19a1b) expression in zebrafish. In a treatment-blinded morphological assessment of larvae at 96 hours post fertilization (hpf), several phenotypes were negatively impacted by BaP, fadrozole (an aromatase inhibitor), and CYP19a1b-morpholino (MO) knockdown including body length, optic vesicle area, swim bladder inflation, pericardial and abdominal edema, and incidence of normal tail shape. In contrast, decreased incidence of normal pectoral fins was caused by BaP exposure only. We hypothesized that co-exposure to estradiol (E2) would rescue the aromatase inhibition-dependent phenotypes. Embryos were exposed to nominal waterborne concentrations of BaP (0, 10 or 50 µg/L), fadrozole (0, 10 or 50 µg/L), CYP19a1b-MO, E2 (10 nM), BaP + E2, fadrozole + E2, or CYP19a1b-MO + E2 for 96 hpf. Whole body larval homogenate E2 concentrations at 48 hpf were significantly decreased by BaP, fadrozole and CYP19a1b-MO treatments. E2 co-treatment rescued mortality mediated by CYP19a1b-MO and the 10 µg/L but not the 50 µg/L concentrations of BaP and fadrozole, suggesting potential AhR-ER cross-talk at the higher concentrations. Adverse developmental outcomes, except non-inflated swim bladders and fin shape, were alleviated by E2 co-treatment further supporting the mechanistic significance of aromatase inhibition in BaP's developmental toxicities. Supported by NIEHS R03 ES018962.

3284 Transgenerational Mitochondrial Toxicity of Benzo(a)pyrene in *Danio rerio*

J. S. Kozal[4], N. Jayasundara[4], C. D. Lindberg[4], A. Massarsky[4], A. N. Oliveri[2,4], E. D. Levin[2,4], J. N. Meyer[4] and R. T. Di Giulio[4]. *[1]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC; [2]Department of Psychiatry and Behavioral Science, Duke University Medical Center, Durham, NC; [3]Integrated Toxicology and Environmental Health Program, Duke University, Durham, NC and [4]Nicholas School of the Environment, Duke University, Durham, NC.*

Mitochondrial integrity is essential to the health and survival of aerobic organisms. Mitochondria are targets of environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs), especially due to metabolic activation by cytochrome P450s and lack of DNA repair mechanisms–mtDNA is maternally inherited and undergoes a bottleneck during early development, potentially leading to transgenerational effects from maternal exposure. To this end, this study evaluated transgenerational effects of the PAH benzo(a)pyrene (BaP) on bioenergetics, mtDNA damage and copy number, oxidative stress, and behavior in the F1 generation following maternal exposure to BaP. Maternal gamete mitochondrial integrity and function were also evaluated. Reproductively mature female zebrafish (F0) were fed 0, 12.5, 125, or 1250 µg BaP/g diet at a feed rate of 1% body weight twice/day for 21 days. The exposed females were bred with naïve males, and the embryos (F1) were collected for subsequent analyses. Maternally exposed (ME) F1 embryos exhibited altered mitochondrial function and metabolic partitioning, measured *in vivo* with the Seahorse XFe24 Extracellular Flux Analyzer. Specifically, ME embryos had lower oxygen consumption rates (OCR) for basal and mitochondrial respiration and ATP turnover, and increased OCR for proton leak and reserve capacity. Dose dependent reductions in mtDNA copy number and increases in mtDNA damage were also measured in ME embryos. DanioVision®, a high-throughput system for tracking zebrafish, revealed that ME larvae were hypoactive relative to controls. Notably, mitochondrial effects in the F1 generation were correlated with bioenergetic impairment in ovaries from the exposed F0 females. Reduced basal and mitochondrial OCR were measured in ovaries *ex vivo* using a novel XFe24 method (also developed for testes). Overall, this study investigates transgenerational mitochondrial toxicity of BaP and presents novel high-throughout tissue bioenergetics analysis methods. This work was supported by NIEHS T32-ES021432 and P42-ES010356.

3285 Developmental Toxicity and AHR Activation of Nitrated and Heterocyclic PAHs

A. Chlebowski[2], W. H. Bisson[2], S. Simonich[2] and R. Tanguay[2]. *[1]Department of Chemistry, Oregon State University, Corvallis, OR and [2]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Nitrated polycyclic aromatic hydrocarbons (NPAHs) and heterocyclic PAHS (HPAHs) are byproducts of combustion and the reactions of PAHs in the environment. NPAHs and HPAHs are present in the environment, yet pose an unknown risk to human health. The developing zebrafish (*Danio rerio*) model was used to evaluate the toxicity of a structurally diverse set of 27 NPAHs and 10 HPAHs. Additionally, their individual activity towards the aryl hydrocarbon receptor (AHR) was evaluated. We assessed all commercially-available NPAHs, as well as two NPAHs synthesized in-house, which are not commercially available, in addition to the 10 commercially available HPAHs. Zebrafish embryos were exposed from 6 to 120 hours post fertilization (hpf) to a dilution series of individual compounds and evaluated for 22 developmental endpoints. AHR activation was determined via CYP1A immunohistochemistry (IHC) as well as a CYP1A reporter zebrafish line, and compared to predicted docking scores from *in silico* AHR models. We observed a range of statistically significant morphological changes, including pericardial and yolk sac edemas, delayed developmental progression, axis malformations, and mortality. Some compounds did not induce observable developmental toxic responses, while others produced concentration-dependent toxicity. The tested compounds also exhibited a range of AHR binding and activation patterns, including expression in the liver, vasculature skin, and yolk. We also compared the developmental toxicity of two amino PAHs (potential metabolites of NPAHs) to the corresponding NPAHs, and observed equal or increased toxicity in the amino PAHs compared to the corresponding NPAHs. This indicates the need for further study of NPAHs and HPAHs as environmental contaminants with the potential for adverse human health effects, as well as further investigation into the mechanisms-of-action of these compounds. Funding: NIEHS Graduate Student T32 Training Grant 3 ES000760, NIEHS P30 ES025128, OSU SRP NIEHS P42 ES016465 (Projects 3 and 5), NIEHS NRSA 1F31ES026037- 01.

3286 Harm-Reduction Tobacco Products Interfere with the Differentiation of Craniofacial Bone

N. R. Sparks[1], M. Bondesson[2] and N. I. zur Nieden[1]. *[1]Department of Cell Biology and Neuroscience and Stem Cell Center, University of California Riverside, Riverside, CA and [2]Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX; [3]Environmental Toxicology Doctoral Program, University of California Riverside, Riverside, CA.*

In utero tobacco exposure is linked to fetal abnormalities including improper development of craniofacial bone. Pregnant women, who cannot refrain from smoking, are often lured to using harm reduction tobacco products (HRTPs), which are marketed as safer alternatives. These products include ultra-filtered cigarettes with reduced carcinogen content, and chewing tobacco, the use of which omits exposure to harmful combustion products. While these HRTPs might decrease cancer risk in the mother, it is unclear whether they act as teratogens, affecting the development and differentiation of bone tissue. Using *in vitro* pluripotent stem cell models and zebrafish embryos, we provide here the first evidence that HRTEs are teratogenic to the developing craniofacial skeleton. *In vitro*, subtoxic HRTP doses decreased osteoblast differentiation in differentiating human embryonic stem cells, which derive osteoblasts primarily from the neural crest (NC) lineage. In exposed zebrafish the precursors to the NC-derived basibranchial, ceratobranchial and hypobranchial bones were completely absent. Mechanistically, HRTPs increased NADPH oxidase activity and reactive oxygen species levels, concomitant with diminished superoxide dismutase and catalase activity, while co-administration of antioxidants reversed inhibition of osteoblast differentiation. HRTPs also decreased the expression of FOXO1/3a, transcription factors regulating free scavenging enzymes, both at the mRNA and nuclear protein activation level. A similar regulation was found for TWIST1, a transcription factor crucial for bone specification and potential target of FOXO1/3a. Together, these data suggest that HRTPs initiate oxidative stress induced disruption of embryonic skeletal development by modulation of transcription factors, which are otherwise necessary for osteoblast specification, production, and protection.

3287 Developmental Toxicity of Polycyclic Aromatic Hydrocarbons in Embryonic and Adult Zebrafish

M. C. Geier, A. L. Knecht and R. L. Tanguay. *Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Polycyclic Aromatic Hydrocarbons (PAHs) are common environmental contaminants that occur in complex mixtures. Many PAHs are known or suspected mutagens and carcinogens, however very little data exists on the potential developmental toxicity of individual PAHs, which is needed to understand the toxicity of environmental mixtures. Additionally, little data exists on whether developmental exposure to PAHs can have effects in later life stages. PAHs were assessed for developmental toxicity using our high-throughput embryonic zebrafish assay. Additionally, each PAH was evaluated for AHR activation, by measuring CYP1A protein expression using whole animal immunohistochemistry (IHC). Responses to PAHs varied in a structurally-dependent manner. High-molecular weight PAHs were significantly more developmentally toxic than the low-molecular weight PAHs. IHC data also showed differential patterns of CYP1A expression, mainly restricted to the vasculature, liver, and skin. High-molecular weight PAHs generally induced more robust CYP1A expression in more tissue types than the lower low-molecular weight PAHs. A subset of PAHs were selected from the list of tested PAHs for evaluating adult effects following developmental exposure. Adult fish were evaluated for learning and oxygen consumption rates. Adult fish developmentally exposed to either benzo[a]pyrene or dibenzo[a,l] pyrene exhibited increased oxygen consumption rates and decreased learning. These data demonstrate the advantages of using a whole animal model to define PAH structure activity relationships and provide a platform for assessing the hazards of individual PAHs and complex PAH mixtures. Partially supported by NIEHS grants # P42 ES016465, T32 ES07060 and P30 ES000210.

3288 TCDD Dysregulates Osteoblast Differentiation in Multiple Cell Lines, and in Japanese Medaka *In Vivo*

A. T. Watson and S. W. Kullman. *Biological Sciences, North Carolina State University, Raleigh, NC.*

Bone and cartilage development have been identified as potentially sensitive targets for TCDD and other aryl hydrocarbon receptor ligands. In this study, we assess how TCDD impacts osteogenesis in the teleost fish Japanese medaka (Oryzias latipes) and MC3T3-E1 mouse pre-osteoblasts. Following embryonic exposure to TCDD, confocal imaging of transgenic medaka larvae (twist1:EGFP, osx:mCherry, col10a1:nlGFP) reveal a significant attenuation of mineralization within vertebral structures, as well as alterations in number and localization of transgene-labeled osteoblast and osteoblast progenitor cells. At the transcriptional level, the osteogenic regulators, osx and runx2, and terminal markers of osteoblast differentiation, opn and bglap, were significantly reduced suggesting that TCDD impacts bone formation *in vivo* through inhibition of key regulators governing osteoblast differentiation. To assess concordance between teleosts and mammalian cells *in vitro*, mouse MC3T3-E1 pre-osteoblasts were exposed to TCDD for 20 days. At early-intermediate stages of MC3T3 differentiation, treatment with 10 nM TCDD resulted in a significant reduction in osx expression. In addition, col1a1 expression and alkaline phosphatase activity were also reduced during this stage when matrix deposition and maturation occur. At terminal matrix mineralization stages, expression of opn and bglap were significantly reduced which suggests a concordant response. Preliminary results with human bone marrow-derived mesenchymal stem cells indicates that TCDD may additionally dysregulate commitment of mesenchymal cells to an osteogenic lineage. Current work is underway to dissect the precise mechanisms whereby TCDD impacts transcriptional regulation of bone formation. These findings demonstrate that TCDD and other AhR ligands present in the environment may significantly impact human bone development, and emphasize the use of small fish as translational models to investigate xenobiotic-induced alterations in skeletogenesis.

3289 Involvement of Dopaminergic System in Spontaneous and Halogenated Flame Retardants-Induced Motor Behaviors in Zebrafish Embryos

H. Teraoka, M. Fujiwara, D. Nijoukubo, Y. Tanaka and T. Kitazawa. *School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan.*

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are widely used flame retardants, but banned or restricted in many countries as both congeners are persistent, bioaccumulative, and toxic to both humans and other organisms in the environment. However, the mechanism of toxicity is largely unclear. Recently, it was reported that PBDE stimulates motor behaviors of zebrafish embryos by unknown mechanism. In the present study, we studied the effects of PCB (Arochlor1245) and PBDE (PBDE-47) on motor movements of zebrafish embryo. As PBDE did not seem to peneterate chorion, we used decholionized embryos in the present study. They started alternating side-to-side contractions of the tail at 17 hp (tail bending) but gradually switched to a full body contraction by 26 hpf (rotation). PCB and PBDE increased tail bending and rotation in a concentration-dependent manner. The effects were blocked by supplementation of L-tyrosine and L-dopa, substrates for dopamine synthesis. Inhibition of tyrosine hydroxylase, a rate-limiting enzyme of dopamine production, by chemicals and the gene-knockdown (th1) increased motor behaviors. Chemical inhibition of vesicular monoamine transporter (VMAT) and the gene-knockdown also increased motor behaviors that were inhibited by L-dopa supplementation. Supplementation of these substrates also inhibits spontaneous motor behaviors. Additionally, inhibition of tyrosinase, a rate-limiting enzyme in melanin synthesis, by chemicals and the gene-knockdown inhibited spontaneous and both retardants-induced motor behaviors. Our results suggest that dopaminergic activity negatively regulates spontaneous and both haloganated retardants-induced motor behaviors in zebrafish embryos. Tyrosinase may competete with tyrosine hydroxylase for tyrosine to affect early dopamine synthesis.

3290 An Adverse Outcome Pathway for the Interaction Between Selenomethionine and Osmotic Stress in Japanese Medaka (*Oryzias latipes*) Embryos

A. Kupsco and D. Schlenk. *Environmental Sciences, University of California-Riverside, Riverside, CA; Environmental Toxicology, University of California-Riverside, Riverside, CA.*

Activities that perturb naturally seleniferous soils, such as mining and agriculture, cause the release of selenium into waterways. Although selenium is an essential micronutrient, it has demonstrated embryo toxicity to fish. Selenomethionine (SeMet) is an organic form of selenium often passed to developing embryos through maternal transfer. Non-chemical stress alters detoxification strategies of developing organisms. We have previously demonstrated potentiated SeMet developmental toxicity in Japanese medaka undergoing concomitant osmotic stress treatments. We previously identified SeMet exposures at stage 25 (48 hours post fertilization) for 24 hours as the most susceptible stage to the osmotic stress potentiation of SeMet toxicity. The objectives of this study were to investigate the molecular mechanisms of these effects to generate an Adverse Outcome Pathway for the interactions between SeMet and osmotic stress (AOP). The unfolded protein response and apoptosis were examined in Japanese medaka (*Oryzias latipes*) embryos treated with 2.5μM SeMet in freshwater and in hyperosmotic conditions beginning at stage 25 and lasting 24 hours. SeMet treatment resulted in approximately 25% mortality in both freshwater and hyperosmotic conditions, yet caused significantly less deformities in freshwater than hyperosmotic conditions (23% compared to 52%). Apoptosis was measured with acridine orange in order to image the localization of these effects. qPCR of downstream UPR targets (CHOP, EDEM1, PDI4a and Dnajb9) was analyzed to assess UPR induction. A significant increase in apoptotic cells was observed in the trunk and tail of embryos treated with SeMet in freshwater and hyperosmotic conditions (152 and 189 cells, respectively, in comparison to 50 and 60 cells in freshwater and hyperosmotic controls). Embryos that exhibited increased malformations following treatment exhibited significantly more staining than embryos that appeared healthy. In contrast to earlier studies, differences were not observed in the expression of UPR transcripts. These data indicate stage dependent changes in the AOP of SeMet under hyperosmotic conditions.

3291 Perfluorooctanesulfonic Acid (PFOS) Alters Pancreatic Organogenesis and Embryonic Redox Signaling in the Zebrafish, *Danio rerio*

K. E. Sant, H. Jacobs and A. R. Timme-Laragy. *Environmental Health Sciences, University of Massachusetts Amherst, Amherst, MA.*

Perfluorooctanesulfonic acid (PFOS) is a persistent environmental toxicant previously found in non-stick products such as Teflon and Scotchgard. PFOS has been found almost universally in human biological samples, including amniotic fluid. PFOS has been associated with increased risk for diabetes, obesity, and metabolic disease, though the underlying mechanism is unknown. Many studies have investigated these associations during adolescence or adulthood, yet very little is known about the role of embryonic exposures in the developmental origins of diabetes. The objective of this study was to determine whether PFOS exposure during organogenesis affects pancreatic beta cell growth and physiology. Embryos from transgenic insulin-GFP zebrafish were exposed to PFOS at 0, 16, 32 or 64 μM concentrations beginning at 3 hpf, and exposures were maintained until 7 dpf, when the pancreas is matured and secondary islets have started to form. Total growth and islet morphology were characterized every 24 h during this time frame. Overall growth significantly decreased in a dose-dependent manner at 7 dpf. The effect of PFOS on islet development followed a non-monotonic dose response curve, and secondary islet formation by 7 dpf was decreased following the same non-monotonic dose response curve. Imaging of ROS concentrations *in vivo* revealed a high degree of staining of tissues derived from the endoderm germ layer of the embryos, and was doubled in PFOS-exposed embryos compared to controls. Redox regulation of the glutathione and cysteine redox couples at 72 hpf followed the same non-monotonic curve, suggesting that redox mechanisms may govern islet growth in the embryo. Gene expression of pancreas hormones was performed in larvae at 96 hpf. Concordant with islet data, there was a non-monotonic decrease of insulin expression with increasing PFOS concentration. Expression of the pdx1 transcription factor, responsible for insulin induction, is only increased at the highest PFOS dose, suggesting that it may contribute to the non-monotonic dose response curves observed for islet growth and insulin production. Overall, this work indicates that PFOS exposure impacts ROS and redox signaling during pancreatic organogenesis, and may impact islet physiology and growth.

3292 Butyl Paraben Affects Pancreatic Development in Zebrafish (*Danio rerio*) Embryos

S. E. Brown[2], K. E. Sant[2], L. Zhao[1] and A. Timme-Laragy[2]. [1]*Department of Nutrition, University of Tennessee, Knoxville, TN and* [2]*Environmental Health Sciences, University of Massachusetts Amherst, Amherst, MA.*

Butylparaben (Butyl p-hydroxybenzoic acid) is a widely used cosmetic and pharmaceutical preservative that has been recently shown to induce oxidative stress and have endocrine disrupting effects in rodents, and promote adipocyte conversion of human adipose cells. Embryonic development is extremely sensitive to oxidative stress due to changes in cell growth, development and differentiation that occur during this life stage. Fluctuations in redox potentials play critical roles in normal embryonic development by guiding these cell signaling, cell-fate decisions and apoptosis. The most prevalent endogenous antioxidant that defends against oxidative stress is glutathione (GSH), which scavenges reactive oxygen species that oxidize protein thiols. The low antioxidant capacity of pancreatic β cells suggests that they are sensitive target tissues of oxidative stress; this has yet to be investigated during embryonic development. Here, we aim to 1) determine whether embryonic exposure to butylparaben prompts structural and functional changes in the developing endocrine pancreas and 2) determine the spatio-temporal mechanism by which butylparaben induces oxidative stress in the developing zebrafish. Transgenic insulin-GFP zebrafish embryos were treated with a sub-chronic exposure to 0.25, 0.5, 1, and 3 μM butylparaben beginning at 3 hours post fertilization (hpf). Pancreatic islet and whole embryo morphological development were examined daily until 7 days post fertilization. Redox potentials were measured at 24 and 28 hpf using HPLC. Area of the pancreatic islet increased over time with butylparaben exposure in a dose-dependent manner by as much as a 68% compared to the controls. Butylparaben concentrations of 0.5 and 1 μM increased GSH by 10 and 40%, respectively, and decreased oxidized glutathione disulfide by 37 and 59%. GSH redox potentials became more reduced with 0.5 and 1 μM butylparaben exposure, decreasing redox potentials by 7 and 18 mV, respectively. Cysteine redox potentials also became more reduced, decreasing by 17 and 28 mV. These data show that butylparaben exposure caused redox potential disruptions that may contribute to the effects on pancreatic islet structure, but further studies are needed to determine how and if that directly affects pancreas development.

3293 Role of Nrf1 Paralogs in Regulating the Transcriptional Response to Phthalates in Zebrafish (*Danio rerio*)

N. Tran[1], L. M. Williams[1] and A. R. Timme-Laragy[2]. [1]*Biology, Bates College, Lewiston, ME and* [2]*Environmental Health Sciences, University of Massachusetts Amherst, Amherst, MA.*

Phthalates, including di(2-ethylhexyl) phthalate (DEHP), are ubiquitous plasticizers that are known to cause oxidative stress and disrupt the endocrine system. While the metabolite of DEHP, mono(2-ethylhexyl) phthalate (MEHP), is consistently detected in human samples, including infants, little is known about the effects of phthalate exposure during early embryonic development. One potential meditator of phthalate toxicity is the nuclear factor erythroid 2-related factor-1 (Nrf1), a protein that is part of the cap 'n' collar basic leucine zipper transcription family. In this study, the zebrafish model was used to assess the role of Nrf1 paralogs in the embryonic response to chronic phthalate exposure. Wildtype, Nrf1a morphant, and Nrf1b morphant embryos were exposed to 200 μg/L mono-(2-ethylhexyl)-phthalate (MEHP) or vehicle (DMSO) control from early development (2 hpf) until their larval stage (96hpf). Morphological changes were noted every 24 hours and the expression of genes related to glutathione (gsr, ggt1b, gstp1, gclc) and oxidative stress (hsp70) were assessed at 96 hpf. MEHP exposure caused morphological changes in all genotypes throughout early development including yolk sac and pericardial edema, melanocyte deficiency, and underdevelopment of the tail. There was no significant difference in the type or severity of morphological changes between wild-type and Nrf1 morphant groups. At the gene expression level, there were no significant changes to glutathione related genes between control and MEHP treated embryos of all genotypes. However, exposure to MEHP did significantly up-regulate hsp70 in wildtype embryos and that up-regulation was Nrf1b dependent. Overall, these data indicate that chronic MEHP exposure does negatively affect embryonic development and potentially causes changes to genes involved in the oxidative stress response that may be mediated via the Nrf1b paralog.

3294 Mono-2-Ethylhexyl Phthalate (MEHP) Alters Embryonic Growth and Pancreatic Organogenesis in Zebrafish

H. M. Jacobs[2], K. E. Sant[2], L. M. Williams[1] and A. R. Timme-Laragy[2]. [1]*Bates College, Lewiston, ME and* [2]*Environmental Health Sciences, University of Massachusetts Amherst, Amherst, MA.*

Di(2-ethylhexyl) phthalate is a persistent environmental contaminant extensively used as a plasticizing agent in PVC piping and medical tubing. It is quickly hydrolyzed in tissue to its primary metabolite, mono(2-ethylhexyl) phthalate (MEHP). MEHP is associated with metabolic disorders such as obesity, and developmental abnormalities. However, little research exists on the effects of MEHP exposure during early embryonic development. Oxidative stress is associated with physiological and morphologic defects in the developing embryo. MEHP is known to induce oxidative stress, which may contribute to abnormal embryonic development. Nrf2 is a transcription factor responsible for the upregulation of the innate antioxidant response. The objective of this study was to determine whether MEHP exposure during early organogenesis results in oxidative stress and may impact embryonic growth and pancreatic β cell development. Wild-type and Nrf2a mutant zebrafish embryos served as a model to test whether MEHP exposure is producing these effects via induction of oxidative stress. Embryos were exposed to 0 or 0.7 μM concentrations of MEHP beginning at 3 hours post-fertilization (hpf), and maintained every 24 hours until 96hpf. A morphology assessment was conducted every 24 h to assess embryo growth and development. Mild to severe pericardial edema was the most common defect observed in both genotypes exposed to MEHP compared to controls. There was no significant exacerbation of effect in the Nrf2a mutant exposure group (28%) compared to the wild-type (32%). Other defects observed in the exposed groups included craniofacial and tail deformities. To ascertain whether these effects were due to oxidative stress, glutathione and glutathione redox potentials were quantified at 72hpf. However, there were no significant changes observed. To assess disruption of pancreatic organogenesis, embryos from transgenic insulin-GFP zebrafish were exposed following the same paradigm, and islet morphology was assessed and the area measured every 24 h. MEHP exposure significantly decreased pancreatic islet area compared to the controls at all timepoints (24, 48, 72, 96hpf). These data indicate that early embryonic exposure to MEHP may negatively impact embryonic growth and pancreatic organogenesis.

3295 Characterization of Heme Oxygenase and Biliverdin Reductase Expression During Zebrafish Development

M. J. Jenny and A. Holowiecki. *Biological Sciences, University of Alabama, Tuscaloosa, AL.*

Although heme molecules are essential for life, unbound heme can generate damaging reactive oxygen species. Two isoforms of the heme-degrading enzyme, heme oxygenase 1 and 2 (HO-1 and HO-2), catalyze the breakdown of heme into biliverdin, which can be further reduced to bilirubin by biliverdin reductase (BVRa and BVRb). Furthermore, it has been suggested that biliverdin/bilirubin recycling functions to protect cellular lipophilic components from oxidative stress similar to the classic glutathione recycling pathway. Multiple techniques were used to fully characterize developmental expression of these enzymes. Real-time RT-PCR identified significant differences in the constitutive expression of HO-1 (1a and 1b) and HO-2 (2a and 2b) paralogs during development. However, both HO-1a and HO-1b isoforms were inducible by pro-oxidants (Cd, t-BHQ, hemin) in zebrafish larvae. Interestingly, only BVRb, and not BVRa, was inducible in larvae by pro-oxidant exposure. *In situ* hybridization (BVRa and BVRb) and *in vivo* promoter analysis (HO-1a) were used to characterize the spatial and temporal gene expression patterns during development. All three enzymes displayed expression patterns consistent with hematopoietic markers regulated by GATA-1, the master regulator of erythropoiesis, during normal development. Interestingly, BVRa and BVRb expression was lost in GATA-1 morphants during early development (20-32 hpf), while mCherry expression driven by the HO-1a promoter appeared to be unaffected. Targeted knockdown of NRF2a, the master regulator of oxidative stress, resulted in loss of Cd-induced HO-1a expression as detected by ISH in older larvae (96 hpf) although mCherry expression remained in hematopoietic tissues in early embryos (24 hpf). Although BVRb expression is induced by pro-oxidants, the spatial and temporal expression was unaffected in older NRF2 morphants (96hpf) but appeared to be enhanced in hematopoietic tissues at 24 hpf. This data provides new insight into the differential regulation of HO and BVR expression during zebrafish development and in response to oxidative stress. [R00ES017044]

3296 Sublethal Effects of Amoxicillin on the Development of Zebrafish (*Danio rerio*)

G. A. Oliveira[1], L. Brito[1], R. Oliveira[2], L. Rodrigues[1], C. Lisboa[2], B. Ferraz[2], N. Nunes[2], C. Grisolia[2] and M. Valadares[1]. [1]*Federal University of Goias, Goiania, Brazil and* [2]*University of Brasilia, Brasilia, Brazil.* Sponsor: D. Oliveira.

Introduction: Amoxicillin (AMX) is a ß-lactam antibiotic widely used to treat or prevent bacterial infections in humans and animals. Nowadays, there is an increasing concern about residues of pharmaceuticals in the environment. AMX has been often detected in effluents of sewage treatment plants, surface waters and sediments. Recent studies have shown that AMX is not acutely toxic to zebrafish (*D. rerio*), with no significant mortality recorded for concentrations < 100 mg/L. However, malformations and biochemical alterations were reported in zebrafish embryos exposed to AMX. Objective: Evaluate lethal and sub-lethal effects of AMX on zebrafish life-stages after for 7 days. Methodology: Fish Embryo Toxicity test based on OECD 236 was performed with modifications. Groups of 20 embryos were exposed in triplicate to a negative control and eight concentrations of AMX (0.75; 1.5; 3.0; 6.25; 12.5; 25; 50 and 100 mg/L). The test was performed in climate chambers at 26 ± 1.0°C with a 12:12 h light:dark cycle for 7 days. Parameters such as mortality, developmental abnormalities, size and biochemical biomarkers were evaluated. Results and discussion: No significant mortality or developmental abnormalities were found in zebrafish embryo exposed to AMX. Significant increase in the larvae size were found at concentrations of 6.25 to 25 mg/L. Effects of AMX on GST, LDH, CAT were assessed and compared, suggesting changes in different metabolic pathways due to AMX exposure. Conclusion: Aquatic concentrations of AMX is < 10 µg/L. Thus, considering our results, short-term effects on wild fish populations are unlikely to occur. Further, studies focusing on changes in microbial gut flora in fish and presence of resistance genes might be a next step for a better understanding of the potential effects of antimicrobials on aquatic life. Financial Support: FAPEG, CAPES and CNPq.

3297 Alterations in Neuro- and Interenal Steroidogenic Responses in the African Sharptooth Catfish (*Clarias gariepinus*) Exposed to Di-(2-Ethylhexyl) Phthalate

A. O. Adeogun[2], O. R. Ibor[2], R. A. Omiwole[2], E. D. Omogbemi[2] and A. Arukwe[1]. [1]*Department of Biology, Høgskoleringen 5, N-7491, Norwegian University of Science and Technology, Trondheim, Norway and* [2]*Department of Zoology, University of Ibadan, Ibadan, Nigeria.*

Adverse reproductive outcomes due to altered steroidogenesis have been linked to inhibition/reduction in the expression of various key enzymes involved in cholesterol uptake/ transport and downstream synthesis in vertebrates. In the present study, we have investigated the hypothesis that exposure to di-(2-ethylhexyl) phthalate (DEHP) will alter organ-specific steroidogenic responses in fish. Juvenile *Clarias gariepinus* were exposed to waterborne DEHP at 0 (control), 10, 100, 200, and 400 µg/L concentrations for 14 days. Alterations in brain and head kidney steroidogenic enzyme gene expressions for StAR, P450scc, cyp17, 3β-, 11β-, 17β- and 20β-hydroxysteroid dehydrogenase (hsd) and 17β-hydroxylase (17β-OHase) were analyzed using RT- qPCR. In addition, kidney StAR and P450scc protein levels were analyzed using immunochemical method with specific antibodies. We observed alterations in mRNA expression of key steroidogenic enzyme genes that were concentration- and time-specific responses. Generally, these responses were biphasic, increasing significantly at 10 µg/L compared to control group, and decreased thereafter in an apparent concentration-specific manner, between the 100 - 400µg/L concentration for most variables. Immunoblotted StAR and P450scc proteins revealed detectable protein cross-reactions at all concentrations with the 10µg/L expressing higher protein band intensities compared to other concentrations. Interestingly, we observed a higher kidney sensitivity (expressed as copy numbers) to DEHP exposure in all examined steroidogenic enzyme genes, compared with the brain. Our findings demonstrated that exposure to DEHP produced alterations in neural and interenal steroidogenesis with probable negative consequences on reproduction and overt physiology for *C. gariepinus*.

3298 Evaluation of Reproductive and Endocrine Toxicity of Boric Acid in the African Clawed Frog (*Xenopus laevis*)

D. Fort[1], T. Fort[1], M. Mathis[1] and R. W. Ball[2]. [1]*Fort Environmental Laboratories, Stillwater, OK and* [2]*Rio Tinto Minerals, Greenwood Village, CO.*

The potential reproductive and endocrine toxicity of boric acid (BA) in the African clawed frog, *Xenopus laevis*, was evaluated following a modified Amphibian Growth and Reproduction Assay (AGRA) model consisting of a 30-d exposure of adult frogs. Adult female and male frogs established as proven breeders were exposed to a culture water control and four target (nominal) test concentrations (5.0, 7.5, 10.0, and 15 mg boron (B)/L, or 28.5, 42.8, 57.0, and 85.5 mg BA/L) using flow-through diluter exposure system. Eight adult female and eight adult male frogs were assigned to each of two replicates per treatment and maintained by sex. Four frogs from each replicate aquarium (8 female and 8 male) were bred with unexposed frogs. The primary endpoints measured were adult survival, growth (weight and snout-vent length [SVL]), necropsy data, reproductive fecundity, and development of progeny (F1) from the exposed frogs. Necropsy endpoints included gonad weight, gonado-somatic index (GSI), ovary profile (oocyte normalcy and stage distribution), sperm count and dysmorphology. Endocrine endpoints included plasma estradiol (E2), testosterone (T), dihydrotestosteone (DHT), gonadal CYP 19 (aromatase), and gonadal5α-reductase (5-AR). Assessment of reproductive fecundity involved evaluation of secondary sex characteristics and breeding success. Measurement of breeding success included number of eggs laid, fertilization, necrosis, and embryo-larval viability (survival and normalcy of development). BA exposure to adult female *X. laevis* increased the proportion of immature oocytes (< stage II) in the ovaries of females, reduce sperm counts and increased sperm cell dysmorphology frequency in male frogs exposed to 15 mg B/L. No effects on the other general, developmental (F1), or endocrine endpoints were observed. Based on the results of the present study, the no observed adverse effects concentration (NOAEC) for the reproductive endpoints was 10 mg B/L; and 15 mg B/L for reproductive fecundity, F1 embryo larval development, and endocrine function. These results confirmed that although BA is capable of inducing reproductive toxicity at high concentrations, it is not an endocrine disrupting agent.

3299 Combination of a Human Pluripotent Stem Cell and Zebrafish Assay to Predict the Developmental Toxicity of Chemicals

J. A. Palmer[2], C. Quevedo[1], L. A. Egnash[2], I. Irijalba[1], A. Muriana[1] and E. L. Donley[2]. [1]*BBD BioPhenix-BIOBIDE, San Sebastian, Spain and* [2]*Stemina Biomarker Discovery, Madison, WI.*

The primary alternative *in vitro-ex vivo* assays for evaluating developmental toxicity potential are cellular models using pluripotent stem cells and models that use embryos. Development of innovative strategies to screen toxicity aimed at reducing or replacing the use of animal models in compound safety testing is critical to meet the safety requirements for multiple industries. Additionally, worldwide initiatives to screen thousands of chemicals currently in use for toxicity potential, such as REACH and Tox21, require inexpensive and high throughput alternative models to meet their goals. Assays developed should allow selection and prioritization of the most promising compounds for further development and effectively reduce the number animals required for developmental toxicity testing. Given the numerous complex processes involved in fetal development, evaluating a test agent in more than one alternative assay yields a more complete view of the test compound's developmental toxicity potential. In this study, twenty-five compounds with varying mechanisms of toxicity (12 non-developmental toxicants, 13 developmental toxicants) were tested in two developmental toxicity assays, the human pluripotent stem cell (hPSC)-based targeted biomarker assay and the zebrafish embryotoxicity test (ZET). For the hPSC-based targeted biomarker assay, human induced pluripotent stem cells were exposed to 8 concentrations of each compound. Spent media was collected to determine changes in biomarkers of developmental toxicity (ornithine and cystine) and cell viability was measured. For the ZET, embryos at 2-4 days postfertilization were treated with 8 concentrations per compound. The presence of functional and morphological alterations was evaluated after 2 and 4 days. A compound was considered to be a developmental toxicant if it had a positive outcome in one of the assays. Combining the results yields ~10-20% increase in sensitivity (detection of developmental toxicants) than when the assays are used alone. Combining the hPSC-based targeted biomarker assay with the ZET joins the advantages of using a human-based model for selection with the determinations of hazards based on molecular and morphological alterations in a whole embryo.

3300 ToxCast Chemical and Bioactivity Profiles for *In Vitro* Targets in the Retinoid Signaling System

N. C. Baker[1], S. Hunter[2], J. A. Franzosa[2], A. Richard[2], R. Judson[2] and T. Knudsen[2]. [1]*Lockheed Martin, Research Triangle Park, NC and* [2]*NCCT, Environment Protection Agency, Research Triangle Park, NC;* [3]*NHEERL, Environment Protection Agency, Research Triangle Park, NC.*

A predictive model for prenatal developmental toxicity using ToxCast Phase I showed the RAR assay set to be the strongest weighting factor (Sipes et al. 2011). Retinoid signaling mediates growth and differentiation of the embryo. ToxCast has 6 reporter assays for trans-activation of retinoic acid receptors (RARa, RARb, RARg, and RXRa, RXRb, RXRg) and cis-activation of DR5 response elements by RAR/RXR, yielding a total of 879 ToxCast AC50s (conc. 50% effect) mapped to molecular targets in the retinoid system. In total, 97 of 1858 chemicals (5.2%) hit ≥ one assays in the retinoid system at an AC50 below 2 uM. Several persistent organochlorines activated the RAR system at AC50s below 2 uM; several organotins and tert-butyl compounds activated the RXR system below 0.2 uM and 2 uM, respectively. All-trans retinoic acid was the most potent chemical tested in the RARa (AC50 = 0.43 nM) and RXRa (AC50 = 0.31 nM) trans-activation assays, and closely followed several tributyltin compounds as the most potent on the DR5 (AC50 = 6.26 nM) assay. A subset of 263 chemicals was tested on mouse embryonic stem cell (mESC) differentiation and cytotoxicity. In all 54% of the subset showed effects on mESC, including 58% of the DR5 compounds, 63% of hCYP1A1 compounds and 81% of RAR/RXR compounds. A preliminary analysis with the devTOXquickPredict (Stemina) ESC assay revealed a weak correlation with chemicals active on the RAR/RXR/DR5 system and a targeted biomarker response (ORN/CYSS ratio) that is ~84% predictive for teratogenicity in a human system. In conclusion, *in vitro* profiling of retinoid signaling identified ~ 5% of ToxCast chemicals with a potential for disruption of retinoic acid signaling through trans-activation of RAR or RXR at submicromolar concentrations. The potential for RAR and RXR to heterodimerize with different nuclear receptor families suggests these compounds may potentially disrupt multiple signaling pathways. This abstract does not reflect US EPA policy.

3301a High-Content Imaging for Predictive Toxicology: Discriminating between Adverse and Adaptive Outcomes

I. Shah[1] and B. van de Water[2]. [1]*US EPA, Research Triangle Park, NC and* [2]*Leiden University, Leiden, Netherlands.*

High-content imaging (HCI) is a cellular systems approach to cataloguing the biological effects of chemicals. Using multi-parametric analysis, HCI can measure integrated cellular responses following the activation or deactivation of multiple molecular functions at the single cell level. Because HCI measures multiple cytological features of fluorescently labeled cells, it provides unique quantitative insight into their phenotypic state in terms of stress, injury, recovery, and death. With technological advancements in creating fluorescently labeled markers, multiresolution imaging, and intelligent image analysis tools, it is feasible to generate HCI data for thousands of chemical perturbations. These complex and large-scale HCI data streams can be interpreted using new informatics and systems biology-based mathematical modeling tools. This makes HCI a powerful *in vitro* tool for predicting the adverse effects of chemicals, a key need in realizing the vision of toxicity testing in the 21st century. This symposium will bring together cutting-edge concepts on HCI as an alternative testing paradigm for predictive toxicology and will focus on 1) innovative tools for interrogating the molecular and cellular state of cells; 2) evaluating sentinel stress response pathways that can profile the chemical-induced perturbations in cells; 3) using HCI responses to analyze the balance between cellular injury and recovery through homeostatic mechanisms; 4) applying HCI data to predict drug-induced liver injury (DILI); and 5) computational systems-level analysis of HCI data to identify toxicological tipping points. This symposium will address these issues in a series of highly focused presentations on 21st century toxicology using HCI as an innovative tool for risk assessment, and for estimating points of departure for risk assessment. This symposium will be of wide interest to SOT members, including scientists working in the regulatory arena as well as those interested in the application of molecular and systems biology to risk assessment. A key challenge to using *in vitro* data in risk assessment is differentiating between adaptive and adverse cellular responses. To further investigate the balance between adaptation and adversity, we studied the effects of hundreds of chemicals in HepG2 cells using HCI. HCI assays were used to measure dose and time-dependent perturbations in p53, JNK, oxidative stress, cytoskeleton, mitochondria, and cell cycle progression. We analyzed this multidimensional HCI datastream using a novel computational model, which interpreted the dynamic response of the HepG2 system to chemicals as cell-state trajectories. The cell-state trajectories produced by different concentrations chemicals describe a range of rich responses of the HepG2 system in many cases. Further analysis of trajectories identified dose-dependent transitions in system recovery, which we call tipping points. The critical concentration of chemicals that produce tipping points was generally much lower than the concentration that produced cell loss. We believe that HCI can be used to reconstruct cell state trajectories, and provide insight into adaptation and resilience for *in vitro* systems.

3301b A HCI Cellular Stress Response GFP-Reporter Platform for Chemical Safety Assessment

B. van de Water. *Leiden University, Leiden, Netherlands.*

To increase our understanding of chemically-induced adaptive stress response pathway activation and its contribution to safety assessment a time-resolved, sensitive and multiplex readout of chemical-induced toxicological relevant cellular stress responses is essential. For this we developed a platform containing a wide panel of distinct adaptive stress response fluorescent protein reporter cell lines. These are used for automated high content live cell imaging and quantitative multi-parameter image analysis to elucidate critical adaptive stress response pathway activation that can contribute to human chemical safety assessment. To conserve the endogenous gene regulatory programs, we tag selected reporter target genes with GFP using BAC-transgenomics approaches. Here we demonstrate the functionality and application of individual BAC-GFP pathway in toxicity reporter cell lines in high throughput 2D and 3D culture models in chemical safety assessment as well as integration of the quantitative data in systems biology-based mathematical modelling to discriminate adaptive from adverse outcome.

3302 Imaging 3D Human Microtissues to Modernize Toxicity Testing

K. Boekelheide. *Brown University, Providence, RI.*

Cells grown in 3D more closely mimic the differentiated function, phenotype, and biology of tissues than the same cells grown in conventional 2D monolayers, because of increased cell-cell contact, enhanced cell-ECM signaling, and the absence of an unnaturally stiff and highly adherent plastic dish. Microtissues suitable for high throughput testing must be simple and highly robust, conditions met by 3D multicellular spheroids formed using micro-molded agarose hydrogels. Using this approach, MCF-7 cells self-assemble and organize into 3D microtissues containing luminal spaces. After 7 days of growth, transmission electron microscopy of the luminal lining cells demonstrates polarization, with the inner apical surface displaying microvilli and secretory vesicles reminiscent of the *in vivo* morphology of the mammary gland epithelium. To test the effects of estrogenic endocrine disrupting compounds (EDCs) on the phenotype of MCF-7 microtissues, samples were exposed to vehicle control (DMSO), the estrogen receptor alpha (ERa) selective agonist propylpyrazoletriol (PPT), or estradiol (E2) for 7 days. Vehicle control 3D MCF-7 microtissues displayed luminal spaces surrounded by 1-3 cell layers of differentiated cells. The morphological alterations were quantified for a PPT concentration-response by determining the ratio of luminal area to total

3303 Integration of High-Content Imaging for Predicting Drug-Induced Liver Injury

W. Tong. *US FDA/NCTR, Jefferson, AR.*

Drug-induced liver injury (DILI) remains a major problem that is largely unpredictable. We have established the Liver Toxicity Knowledge Base to integrate a diverse set of quantitative data on DILI both from pre-clinical data as well as patient-derived information. Using computational methods we integrate this information to derive strategies to better predict human DILI. This involves both structure activity relationships, toxicogenomics data as well as HCI data. The latter dataset involves the exposure of cryo-preserved primary human hepatocytes (PHH) exposed to a large panel of DILI compounds followed by HCI of oxidative stress, mitochondrial damage and cell death. We will describe how this data contributes to DILI prediction and its comparison to other *in silico* predictive tools for DILI.

3304 Using High-Content Imaging to Analyze Cellular Tipping Points

I. Shah. *US EPA/NCCT, Research Triangle Park, NC.*

A key challenge to using *in vitro* data in risk assessment is differentiating between chemical-induced adaptive versus adverse cellular responses. To further investigate this issue, we studied the effects of hundreds of chemicals in HepG2 cells using high-content imaging (HCI). HCI measured chemical dose and time-dependent perturbations in p53, JNK, oxidative stress, cytoskeleton, mitochondria, and cell cycle progression. We developed a novel computational model to analyze these multidimensional HCI datastreams, and used this model to interpret the dynamic responses to chemicals as cell-state trajectories. By analyzing trajectories for each chemical we found three concentration-dependent trends in HepG2 cell behaviors including: (a) adaptation followed by complete recovery, (a) adaptation followed by partial recovery, and (c) adaptation without recovery leading to irreversible injury. We believe that the concentration-dependent transition from adaptation to injury is a "tipping point" for the system. Tipping points are generally observed at chemical concentrations far below cytotoxic levels. We believe that rich HCI data for chemicals can be interpreted with computational models to find tipping points, and gain unique insight into the threshold for homeostatic adaptation. With additional work, tipping points could also be used as the point of departure for risk assessment.
This abstract does not represent US EPA policy.

3305 Utilizing High-Content Imaging Tools Toward Pathways-Based Safety Assessments: A Consumer Product Industry Perspective

P. Carmichael. *Unilever, Sharnbrook, United Kingdom.*

Eliminating the need to use animal data in the safety assessment of new ingredients intended for use in consumer products has been a long and deep set ambition for Unilever. The biggest challenge is posed by the traditional use of animals for systemic (internal organ based) toxicology testing which is embedded in regulatory guidelines worldwide. Since 2007 and the inspiration of the US National Academy of Science's report 'Toxicity Testing in the 21st Century: a Vision and a Strategy', Unilever safety scientists have been seeking not to replicate each animal test with an 'alternative' but to capitalize on the recent revolution in biomedical sciences in order characterize toxicological pathway changes in human biological systems. The use of high content imaging (HCI) tools is at the heart of this work and our focus is now on exploiting new mechanistic understanding to craft bespoke exposure-led safety assessments for new ingredients using *in vitro* and *in silico* methods: establishing whether a new agent has the potential (or not) to perturb cellular homeostasis and stress networks beyond their normal restorative capacity. Our work, with various partners in this space, has encompassed a number of areas including adaption/adversity decision-making on DNA damaging agents, on androgen receptor effects, lung fibrosis and as a general tool for early MIE definition. This work fits into our larger strategy of working towards using these tools as part of a TT21C safety assessment. Developing the models and tools to reliably achieve these aims is an on-going odyssey that has grown as new scientific partners from the EU, USA and China have joined to engage regulators with these new approaches to safety assessment while assuring the highest standard of safety for our consumers, workers and the environment.

3306a Novel Roles of Reactive Oxygen Species (ROS) in Human Diseases: Why ROS Never Gets Stale

B. Moorthy[1] and D. Zhang[2]. [1]*Baylor College of Medicine, Houston, TX and* [2]*University of Arizona, Tucson, AZ.*

Oxidative stress mediated by reactive oxygen species (ROS) can have a profound effect on many cellular functions. The major goal of this symposium is to discuss the molecular and cellular mechanisms by which ROS, including free radicals, contribute to oxidative stress and alter various signaling pathways, which could in turn lead to inflammation and cell death in target organs, and ultimately lead to human diseases including chemically induced acute hepatitis, neurodegenerative diseases, and pulmonary diseases such as bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), and cancer. The innovative aspect of the proposed symposium is to discuss the molecular mechanisms of oxidative stress and signaling that leads to inflammation and cell death in a cell- and organ-specific manner, and the mechanisms by which they contribute to target organ toxicities. The recent findings of the novel roles of oxidative stress in multiple human diseases warrant the need for a symposium to discuss the latest mechanistic research in this area and its impact on human health. Specifically, the symposium will discuss (i) molecular signaling pathways that selectively affect SNpc neurons, and how these studies could help unravel the mechanisms underlying selective vulnerability of these neurons in neurodegenerative diseases like Parkinson's disease (PD); (ii) the role of nuclear receptors, ER stress, and oxidative stress in fatty liver disease, and how modulation of FXR activity and the related bile acid metabolism in hepatocytes might be a promising strategy to treat chemically induced acute hepatitis; (iii) the mechanistic role of omega-3 epoxides, which are anti-inflammatory; inhibit angiogenesis, tumor growth, and tumor metastasis induced by TCDD; and, recent studies have suggested, may act as antioxidants; (iv) the molecular role of Nrf2 as master regulator against oxidative stress, its implications for multiple human diseases including cancer, and the discovery of novel small molecule nrf2 modulators to improve human health; and (v) the novel roles of cytochrome P450 (CYP)1A and 1B1 enzymes in hyperoxic lung injury, in relation to BPD and ARDS. In conclusion, this symposium will discuss the latest developments in oxidative stress, molecular signaling, inflammation, and cell death, and offer new opportunities for clinical and translational research.

3306b Introduction

B. Moorthy. *Baylor College of Medicine, Houston, TX.*

The major goal of this symposium is to discuss the molecular and cellular mechanisms by which ROS, including free radicals, contribute to oxidative stress and alter various signaling pathways, which could in turn lead to inflammation and cell death in target organs, and ultimately lead to human diseases including chemically-induced acute hepatitis,

neurodegenerative diseases, and pulmonary diseases such as bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), and cancer. In this symposium, the following topics will be presented: (i) molecular signaling pathways that selectively affect SNpc neurons, and how these studies could help unravel the mechanisms underlying neurodegenerative diseases like the Parkinson's disease (PD); (ii) role of nuclear receptors, ER stress, and oxidative stress in fatty liver disease; (iii) the mechanistic role of omega-3 epoxides, which are anti-inflammatory, and inhibit angiogenesis, tumor growth and tumor metastasis induced by TCDD; (iv) the molecular role of Nrf2 as master regulator against oxidative stress and its implications for multiple human diseases including cancer; and (v) the novel roles of cytochrome P450 (CYP)1A and 1B1 enzymes in hyperoxic lung injury, in relation to BPD and ARDS. This symposium, thus, will provide an overview of the latest developments in the field of ROS and oxidative stress, in relation to organ-specific human diseases.

S 3307 Cell-Specific Activation of Redox-Driven Death Signaling Pathways in Neurodegeneration

V. Ravindranath. *Indian Institute of Science, Bangalore, India.*
Sponsor: B. Moorthy.

Neurodegenerative disorders such as Parkinson's disease (PD), motor neuron disease and Alzheimer's disease are characterized by loss of specific cells within certain regions of the brain leading to selective loss of function. PD is a progressive movement disorder that results primarily from the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Mitochondrial dysfunction, oxidative stress and accumulation of mis-folded proteins represent the principal pathways that underlie the pathogenesis of sporadic PD. Increased oxidative stress can result in reversible and irreversible oxidative modification of cysteine residues in key proteins and altered redox status leading to activation of signaling cascades that determine cell survival versus cell death. Delineating signaling pathways that selectively affect SNpc neurons could help unravel the mechanisms underlying selective vulnerability of these neurons in PD. Using an animal model of PD we demonstrate the redox-driven activation of death signaling cascade in the SNpc neurons leading to nuclear translocation of p53 and downstream events, such as p53 mediated transcriptional activation of Bax expression in the ventral midbrain. Selective inhibitors of the specific SAPK/MAPK prevent translocation of p53 and protect against neuronal cell death in models of PD and, thus may help preserve the surviving neurons and slow down the disease progression in PD.

S 3308 Role of Nuclear Receptors, ER Stress, and Oxidative Stress in Fatty Liver Disease

F. Gonzalez. *National Cancer Institute, Bethesda, MD.*

Fatty liver or non-alcoholic fatty liver disease (NAFLD) can be caused by chronic chemical insult such as alcohol, acute chemical exposure such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and by obesity. NAFLD is characterized by massive ectopic triglyceride accumulation in the liver in the absence of other liver disease, or by chronic alcohol consumption. NAFLD is the most prevalent liver disorder affecting up to 30% of the adult population and more than 80% of obese people. Importantly, NAFLD can develop into nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis that can lead to hepatocellular carcinoma. However, the underlying molecular mechanism of NAFLD pathogenesis remains largely unknown. Recent studies have implicated the gut microbiota in the development of NAFLD in mice and in humans. Oral treatment of lean germ-free mice with the cecal microbiota of obese mice caused an increase in fatty liver. Metagenomic studies in humans, and human to mouse microbiota transplantation studies also suggest a role for gut bacteria in metabolic diseases including NAFLD. Gut bacteria can metabolize bile acids that can either activate or antagonize the intestinal farnesoid X receptor (FXR), and increased FXR signaling can give rise to NAFLD. This is mediated by an FXR-induced elevation in serum ceramides that can cause increased hepatic ER stress that results in fatty liver; inhibition of FXR signaling decreases serum ceramides and reduces hepatic lipid accumulation. In another pathway, TCDD can cause increased hepatic inflammation and elevated oxidative stress that can give rise to NAFLD and NASH. While the precise mechanism for TCDD-induced liver steatosis is not known, the data suggest that Kupffer cell-hepatocyte interactions potentiate this response. These mechanistic studies can lead to chemoprentive and therapeutic strategies for the NAFLD.

S 3309 2,3,7,8-Tetrachlorodibenzo-p-dioxin Markedly Increases the Levels of Highly Bioactive Epoxide Metabolites of Omega-6 and Omega-3 Polyunsaturated Fatty Acids in Many Organs of Mice

O. Hankinson. *University of California, Los Angeles, CA.*

,3,7,8- Tetrachlorodibenzo-p-dioxin (TCDD) adversely affects many mammalian organs and tissues. These effects are mediated by the aryl hydrocarbon receptor (AHR). CYP1A1, CYP1A2 and CYP1B1 are upregulated by the liganded AHR. These (and other) cytochromes P450 have been shown *in vitro* to be capable of metabolizing polyunsaturated fatty acids (PUFA) into a variety of bioactive oxylipin metabolites. We measured the levels of 77 oxylipins in various organs/tissues of C57BL/6 mice treated with TCDD or vehicle by intraperitoneal injection. A solid phase extraction protocol was used to extract the fatty acids. LC/MS/MS analyses were carried out to quantitate the levels of individual oxylipins. We found that TCDD increased the levels of cytochrome P450-dependent epoxide and diol metabolites of the omega-6 PUFAs, arachidonic acid (ARA) and linoleic acid in the serum, liver, lung and spleen of C57BL/6 mice in an aryl hydrocarbon receptor-dependent fashion, and we then showed that TCDD markedly increased the levels of the corresponding metabolites of the omega-3 PUFAs, a-linolenic acid, eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA) in the liver and lungs of mice, in several cases more than ten-fold. The increases in these metabolites correlated with increased levels of CYP1A1, CYP1A2 and/or CYP1B1 elicited by TCDD. The omega-6 and omega-3 epoxides have a variety of biological effects at nanomolar concentrations. For example, they are anti-inflammatory. Of considerable interest, the omega-3 epoxides have been reported to have opposite effects on angiogenesis, tumor growth and tumor metastasis compared with the equivalent metabolites of omega-6 PUFA. Furthermore, the changes elicited by TCDD on the levels of omega-6 and omega-3 epoxides in the liver and lung are in a range known to impact the above parameters of tumor behavior in these organs. Our observations therefore have important implications with regard to the potential role of dietary omega-6 and omega-3 PUFA and their cytochrome P450 metabolites in mediating the biological effects of TCDD and other agonists of AHR.

S 3310 Nrf2: Tumor Suppressor or Oncogene?

D. Zhang. *University of Arizona, Tucson, AZ.*

The Nrf2-Keap1 pathway is a redox sensitive signaling cascade that functions to protect a variety of cell types against oxidative stress, environmental toxicants, and harmful chemicals through the induction of cytoprotective genes. Under strict regulation, cells invest much energy into the maintenance of this pathway to ensure rapid induction upon stressful conditions to achieve redox balance. Therefore, controlled activation of Nrf2 defense response by canonical Nrf2 inducers have been recognized as a means for chemoprevention. On the other hand, constitutive activation of Nrf2, due to somatic mutations of genes that control Nrf2 degradation, promotes carcinogenesis and causes chemoresistance of cancer cells. Furthermore, recent findings indicate a potential tumor-promoting role of the prolonged activation of Nrf2 through a noncanonical p62-dependent mechanism. Recent efforts in the field are to determine if Nrf2 is a tumor suppressor or an oncogene. Many conflicting results have been reported recently. In this talk, I will discuss three modes of Nrf2 regulation: canonical (in a healthy individual, controlled transient activation), non-canonical (p62-autophagy, prolonged activation) and loss-of-control (in cancer cells, constitutive activation). I will share our ongoing studies designed to clarify the role of Nrf2 in tumor initiation and promotion in both chemical and genetic carcinogen models. Finally, I will discuss how to use this knowledge to develop small molecule Nrf2 modulators to improve human health.

S 3311 Mechanistic Roles of Cytochrome P4501A and 1B Enzymes in Oxidative Stress-Mediated Mechanisms Leading to Hyperoxic Lung Injury: Implications for BPD in Infants and ARDS in Adults

B. Moorthy. *Baylor College of Medicine, Houston, TX.*

Hyperoxia is frequently used in the treatment of pulmonary insufficiency in preterm and term infants and in adults with acute respiratory disease (ARDS). In preterm infants, hyperoxia contributes to the development of bronchopulmonary dysplasia (BPD). The molecular mechanisms of oxygen-mediated pulmonary injury are not understood, but reactive oxygen species (ROS) are the most likely candidates. Using *in vivo* approaches entailing Cyp1a1- and Cyp1a2-null, Cyp1a1_1a2-double null, and Cyp1b1-null mice and *in vitro* approaches entailing

purified human CYP1A1/1A2 and LC-MS/MS, we observed novel roles for cytochrome P450 (CYP)1A enzymes in the metabolism of lipid hydroperoxides (e.g., F2-isoprostanes/isofurans), which are generated by ROS-mediated mechanisms. Mice lacking the gene for CYP1A1 and the liver-specific CYP1A2 are more susceptible to hyperoxic lung injury, and these mice displayed augmented levels of pulmonary F2-isoprostanes and isofurans, suggesting that these molecules contribute to hyperoxic lung injury. We also have evidence to suggest that FICZ is the endogenous ligand of the Ah receptor (AHR) that contributes to induction of CYP1A enzymes by hyperoxia. On the other hand, Cyp1b1-null mice are less susceptible to oxygen injury, suggesting that CYP1B1 contributes to hyperoxic injury by generating ROS or that antioxidant enzymes are upregulated by hyperoxia in the Cyp1b1-null mice. Recently, we discovered the formation of bulky oxidative lesions (oxidative DNA adducts) derived from F2-isoprostanes in tracheal aspirates of premature infants and adults who received supplemental oxygen, suggesting that these adducts could serve as novel biomarkers of BPD and ARDS. Future studies could lead to the development of rational strategies for the prevention/treatment of lung diseases associated with hyperoxia.

S 3312a Use of the Adverse Outcome Pathway (AOP) Concept to Link Epidemiological to Mechanistic Data on the Correlation of Pesticide Exposures and Parkinson's Disease

M. Leist[1] and S. Hougaard Bennekou[2]. [1]*University of Konstanz, Konstanz, Germany and* [2]*Danish EPA, Copenhagen, Denmark.*

A recent systematic review of epidemiological studies (EFSA External Scientific report, 2013) concluded that the weight of evidence supports an association between exposure to pesticides and increased risk of Parkinson's disease (PD). Analyses of epidemiological and experimental studies by ANSES (the French Agency for Food, Environmental and Occupational Health and Safety) reached a similar conclusion. Such data have attracted significant public attention, but it is unclear whether and how they can be used for risk assessment. It is possible that mixture effects, long-term low-dose exposure, and species differences contribute to effects observed in the human population that have not been predicted from animal experiments. This also has to be balanced against underlying understanding of this type of disease, which has a multifactorial etiology. Therefore, new approaches of data integration from heterogeneous sources (ranging from epidemiology to molecular and animal studies) are required. The goal of the proposed symposium is to use the Adverse Outcome Pathway (AOP) concept as a novel, mechanistically driven approach to assess the biological plausibility of the epidemiological data. This concept represents a shift toward a knowledge-driven approach to toxicological hazard and risk assessment. It relies on a mechanistic understanding of toxicity pathways and attempts to align different types of information to these pathways. An AOP links information on molecular initiating events (MIE) to a final adverse outcome (AO). It provides a basis for multi-level data integration by defining the intermediate key events (KEs) that link the MIE to the AOP, and providing a scientifically firm rationale for the KE relationships, i.e., processes and their thresholds that lead to the activation of the next KE (considering relevant exposure and internal doses). Thus, AOP provides an ideal basis for a strategy that aims to evaluate the evidence of cause-effect relationships. The potential to combine various AOPs to larger networks allows judgments on mixture effects and complex scenarios, such as the one of pesticide exposure possibly leading to Parkinsonism. During this session, speakers with expertise in epidemiology, regulatory toxicology, and basic science will contribute different perspectives to a large and representative case study in which the AOP format is applied to integrate the epidemiological data with mechanistic research. The ultimate goal is to explore the link between exposure to specific pesticides and the risk of developing PD. Individual pesticides, such as rotenone, paraquat, and organochlorine compounds will be used to demonstrate the basic outline of the strategy. The defined examples will be used to highlight its strengths and challenges. The latter include exposure and toxicokinetics considerations, and, e.g., nonlinear network dynamics resulting from feedback and feedforward loops and pathway intersections. Eventually, the integration of individual AOPs to an AOP network underlying a complex human disease will be illustrated. The session will also include recommendations on how to improve the quality of this information and how to integrate these data into regulatory decision-making.

S 3312b The Risk Assessment of Pesticide-Induced Neurotoxicity (Including Parkinson's Disease) in the Standard Regulatory Framework

S. Hougaard Bennekou. *Danish EPA, Copenhagen, Denmark.*
Sponsor: M. Leist.

In the European pesticide regulation, neurotoxic effects of pesticides are considered of particular importance and therefore neurotoxic pesticides are for example candidates for substitution. Under both the former and current data requirements for approval of pesticides, mandatory endpoints for neurotoxicity are those covered by the relevant OECD guidelines for repeat and single dose studies. However, targeted neurotoxicity testing is required only if neurotoxicity is detected in these studies, or if the pesticide in question has known neurotoxic effects. The data on each pesticide are re-evaluated every 10 years, and for this purpose, all relevant epidemiological data and new publications should be reported to contribute to a new and comprehensive risk assessment. Beyond this evaluation of individual compounds, data can also be collected on compound classes or overall risk linked to general pesticide exposure. This talk will give an overview of the results from two large recent meta-analyses of epidemiologic data that suggested an association between exposure to pesticides and the risk of developing Parkinson's disease (PD). These studies have raised the question of whether the current standard repeat-dose animal studies are i) sensitive enough triggers for more targeted neurotoxicity testing, and ii) whether they are sufficient to detect adverse outcomes of relevance to human neurological disease, such as PD. One of the strategies of the European Food Safety Authority (EFSA) to address the gap between epidemiologic data and standard experimental safety assessment is to explore the AOP concept for both interpretation of epidemiologic data and for guidance on follow-up experimental work. The outline of this approach will be presented.

S 3313 An Overall Mechanistic Map of Parkinson's Disease and Interaction Points for Various Pesticides

M. Leist. *University of Konstanz, Konstanz, Germany.*

Evaluation of neurotoxicity currently relies almost exclusively on alerts created during 28/90-day classical toxicity studies, sometimes followed up by a specific test according to OECD TG424 (rodent neurotoxicity). The apical endpoints of animal studies may lack sensitivity for human-relevant forms of toxicity, including development of Parkinson's disease (e.g. complex functional defects, cognitive disturbances, irritability, altered alertness). A new approach of multi-level data integration may provide a better basis for risk assessment. We suggest and illustrate here a formalized description of a definitive chain of key events (KE) leading to specific adverse outcomes (AO) in man. Such adverse outcome pathways (AOP) allow the assessment of relevant KE both in rodents and in many alternative test systems to provide additional information for hazard assessment. AOP starting from different molecular initiating events (MIE) can lead to the same AOP, thus explaining mixture effects on human health. Moreover, different AO, all belonging to the same human disease (e.g. motor disturbances and cognitive deficits in PD) can be combined in one network of intersecting AOPs. After construction of an AOP, and definition of a network, it can be aligned with very different types of data (e.g. from human pathology, animal experiments and biochemical studies). Thus, the AOP concept provides a scaffold for providing broad and mechanistically plausible evidence for the potential presence or absence of neurotoxicity relevant for humans. The use of AOP-based information-rich data packages puts risk assessment on a firmer and more rational basis. The presentation will first give an overview of a mechanistic PD map. It will then demonstrate the combined use of data obtained from diverse models including: (i) cell based systems; (ii) *in vivo* rodent data; and (iii) human epidemiological data. It will show strategies on how to proceed with risk assessment on the basis of AOP-aligned hazard data sets, information on internal doses derived from PBPK models and exposure information, and how such information on a variety of pesticides can be combined to construct an overall mechanistic map of Parkinson's disease that provides a rationale for interpretation of epidemiological data.

3314 AOP Construction Rules and Key Event Relationships: The Cascade of Key Events Initiated by Inhibition of Complex I and Leading to Parkinson's

A. Price. *European Commission Joint Research Center, Ispra, Italy*.
Sponsor: M. Leist.

In the presentation, the AOP framework will be used to link the key events (KEs) triggered by inhibition of the mitochondrial complex I (Molecular Initiating Event; MIE) to the adverse outcome (AO), e.g., motor deficits characteristic of Parkinson's disease. In the brain, mitochondria play a pivotal role in neuronal and glial cell death because they are regulators of both energy metabolism and apoptotic/necrotic pathways. In rats, chronic systemic complex I inhibition caused by the pesticide rotenone induces PD-like features including selective nigrostriatal dopaminergic lesions and development of alpha-synuclein-positive cytoplasmic inclusions in nigral neurons, which result in hypokinesia and rigidity. Therefore, based on a systematic review of the literature, the following links in the AOP have been identified: MIE: Binding of inhibitor to a subunit of NADH-ubiquinone oxidoreductase; KE1: Partial or total inhibition of Complex I; KE2: Mitochondrial dysfunction; KE3: Impaired protein degradation and damaged intracellular transport; KE4: Degeneration of dopaminergic (DA) terminals in the striatum; KE5: Cell death of DA neurons in substantia nigra (pars compacta) and AO: Symptoms associated with motor deficit. An additional KE, linking to KE3-5 will also be considered: KEx: triggering of 'neuroinflammation'. The proposed AOP is developed in a qualitative and where possible quantitative manner (defining the exact KE relationships) taking into consideration dose, time and route of exposure to support systematic use of mechanistic knowledge for regulatory safety assessment of human health.

3315 Experimental Validation of an AOP: Probing Inhibition of Aldehyde Dehydrogenase (ALDH) as the Molecular Initiating Event Linking Exposure to Organochlorine Pesticides and Dithiocarbamate Fungicides to Parkinson's Disease

P. Lein. *University of California, Davis, Davis, CA*.

Epidemiological data link exposure to organochlorine pesticides and dithiocarbamate fungicides to an increased risk of Parkinson's disease (PD). The exposure to these compounds may occur occupationally, but also in other settings. ALDH inhibition by these compounds may provide a mechanistic link of the epidemiologicval data to the human disease process. This presentation will evaluate the experimental evidence for such a link by constructing an adverse outcome pathway (AOP) in which the molecular initiating event (MIE) is ALDH inhibition. This would then lead to a loss of dopaminergic neurons in the substantia nigra, which is associated with the motor deficits that are the key clinical symptom of PD, i.e. the adverse outcome (AO). The presentation will present the KE of this AOP, and it will examine experimental evidence that the pesticide compounds in question trigger this AOP. Experimental validation of this AOP would allow establishment and use of mechanistically-based assays for higher throughput screening of potential chemical risk factors for PD

3316 Multiple AOP Alignment Case Study: Pathways of Toxicity Induced by Exposure to Paraquat That Cause Symptoms of Parkinson's Disease

E. Fritsche. *Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany*.

A systematic review of molecular mechanisms associated with paraquat-induced Parkinsonism revealed that this compound triggers multiple molecular initiating events (MIEs) that are specific to different cellular structures. Thus, at least 4 to 5 AOPs need to be constructed that link paraquat exposure to symptoms of Parkinson's disease. These MIEs include: (1) interaction with NADH-dehydrogenase (complex I), (2) NADH-cytochrome c reductase (complex III), (3) NADH-quinone oxidoreductase (NQOR), (4) NADPH-oxidases (NOX) and (5) xanthine oxidase, all of which contribute to common key events (KE) that activate redox cycling and associated production of reactive oxygen species. Depending on the cellular location of the MIE, i.e. inner (MIEs 1&2) or outer (MIE 3) mitochondrial membrane, plasma membrane (MIE 4) or cytosol (MIE 5), downstream KEs likely differ, but converge on the organ effects (dopaminergic cell death in the substantia nigra). Such a framework interlinks individual AOPs, which share the same AO, and facilitates identification of KE-based biomarkers for *in vitro* testing purposes. It also allows a new approach to the interpretation of controversial animal data on paraquat neurotoxicity and its relevance to humans. The multiple-AOP approach will use all available data, from both *in vitro* and *in vivo* studies, link the mechanistic data to human exposure, and examine the relevance for development of Parkinson's disease.

3317a Advanced Techniques in PBPK Modeling to Improve Quantitative Risk Assessment for Infants and Children

S. Felter[1] and J. Fisher[2]. [1]*Procter & Gamble, Cincinnati, OH and* [2]*National Center for Toxicological Research, US FDA, Jefferson, AR*.

Methods for quantitative risk assessment most commonly employ uncertainty factors to account for species differences and human heterogeneity, including differences associated with life stages, in both toxicokinetics and toxicodynamics. While this approach is generally considered sufficient to provide protection for more sensitive subpopulations and life stages, it is a very crude approach to risk assessment that does not take into consideration data that are increasingly available to address species differences and population variability. Specifically for life stage-associated differences, it is commonly assumed that infants and children are more sensitive than adults, despite data showing that this is often not true. Further, it is increasingly recognized that differences in sensitivity can change very quickly after the neonatal period such that very young infants might be more sensitive, but older infants and children are less sensitive than adults. Alternatively, for an early-life stage compared to adults, there can be increased sensitivity to one endpoint/target organ while there is decreased sensitivity to another, or the differential sensitivity can be related to dose. These differences are particularly important to understand in pharmaceutical development, where dosing must carefully consider both efficacy and safety over different life stages. The immaturity of key physiological processes associated with the toxicokinetic/toxicodynamic handling of xenobiotics is an important factor contributing to the differential susceptibility associated with early-life exposures; this is made more challenging by the rapid changes that occur in both growth and maturation throughout infancy and childhood. PBPK modeling provides an important tool to describe these key maturational processes such that they are explicitly considered in the risk assessment process. While application of these models generally confirms the adequacy of current default UFs for providing protection for early-life stages, it provides an opportunity to refine this assumption while significantly increasing our confidence in the resulting analysis.

3317b Differential Physiology and Sensitivity of Infants and Children

A. Collier. *University of British Columbia, Vancouver, BC, Canada*.
Sponsor: S. Felter.

The physiology and maturational processes/timelines in the developing infant/child as key parameters in the toxicokinetic handling of xenobiotics is addressed. Emphasis will be on hepatic and renal development as they impact the metabolism and excretion of chemicals. The biochemical development of the major metabolizing enzymes P450, UDP-glucuronosyltransferases (UGTs), and Sulfotransferases (SULTs) show that some enzymes are higher in fetal life (some P450s and Sulfotransferases) while others develop biochemically in the neonatal period generally within the first 2 years (UGTs, some P450s). These biochemical aspects of pediatric sensitivity are coupled with neonatal and pediatric physiological changes including: differences in blood albumin levels, microsomal protein per gram of liver (MPPGL) and changes in liver and kidney volumes and blood flows that occur differentially from 0-2 years of age and then mature during late childhood or adolescence. Lamotrigine and morphine will be used as examples of exposures where neonatal and pediatric toxicity is higher due to differential biochemical development of UGT enzymes, as well as physiological maturation. Acetaminophen toxicity in neonates and infants will be used to illustrate how toxicity can depend on dose, and the implications for testing chemicals at high doses where pharmacokinetic processes may be saturated, resulting in effects that are not necessarily relevant to lower doses. Finally, because human health risk assessments are most commonly based on rodent data, species-specific pathways that exist in redox, glucuronidation and sulfation metabolism as well as species differences in common enzymes with different ontogeny will be compared and contrasted to demonstrate both the utility and limitations of rodent toxicity testing for developmental xenobiotic exposure.

3318 Age-Specific PBPK Models

J. Troutman. *Procter & Gamble, Cincinnati, OH.* Sponsor: S. Felter.

Chemical risk assessment often involves extrapolation of repeat-dose toxicity data from laboratory animal species to establish acceptable exposure limits for humans, with uncertainty factors (UFs) being used to account for potential interspecies differences and inter-individual variability for both toxicokinetic handling and toxicodynamic response to a chemical exposure. PBPK models can be used to refine assessments by replacing default uncertainty factors with chemical-, species- and age-specific data. To address complexities in the available rodent dose-response data for a commonly used preservative, 2-phenoxyethanol (PhE), rat and human PBPK models were developed. In addition, the human model was parameterized with infant-specific data to support a risk assessment for exposure of infants to PhE in cosmetic products. Based on model-predicted differences in the internal concentration-time profiles between oral bolus and drinking water dose regimens in rat toxicity studies, systemic area -under -the plasma concentration-time -curve (AUC) was selected as the appropriate dose metric for comparison to aggregate dermal and oral consumer product use scenarios in both infants and adults. The utility of PBPK modeling will be illustrated with model simulations that allow one to predict internal doses in rats versus and humans and compare this to refine the default UF that would be applied for extrapolation across species in the absence of model-predicted exposures. Similarly, PBPK models can be used to evaluate kinetic differences in humans associated with early life stages. Comparison of the dose-normalized AUC ratio of PhE for the adult and infant is shown to be less than 2-fold, confirming that for this case, the default UF (3.2-fold) that is used to account for human heterogeneity in kinetics is sufficiently protective for early life exposures and can be refined with the use of PBPK modeling. Finally, it is recognized that there is a need for extension of these models to cover premature infants, which represent a particularly challenging life stage to model. Progress of ongoing research to extend PBPK models to premature infants will be discussed briefly.

3319 Advances in Extrapolation to Help Inform Pharmacokinetics and Pharmacodynamics of Chemicals and Drugs in Infants and Children

J. Fisher. *National Center for Toxicological Research, US FDA, Jefferson, AR.*

The development of PBPK/PD models to describe and predict the pharmacokinetics and pharmacodynamics of chemicals and drugs during maturation of infants and children remains a challenge. For infants and children classical scaling of model parameters, such as using BW3/4, usually does not adequately describe the growth of organs or the kinetic constants for enzymes and transporter proteins. Empirical methods are routinely used in place of scaling, if the data exist. The US FDA Best Pharmaceuticals for Children Act (BPCA) has resulted in a ground swell of published PBPK models for drugs and children. However, few published papers report on the development of PD models for early life stages. This presentation provides computational insights into the maturation of the thyroid endocrine system for infants and discusses the potential impact of chemicals or drugs on this endocrine system. PBPK/PD modeling of the HPT axis in the lactating mother-nursing infant pair is presented to demonstrate non-thyroid stimulating hormone (TSH) compensatory processes used by the mother and infant to cope with moderate iodine deficiency. Iodine is an essential nutrient that required for normal development.

3320 Life Stage Physiologically-Based Pharmacokinetic (PBPK) Model Applications to Screen Environmental Hazards

H. El-Masri. *National Health and Environmental Effects Research Laboratory, US EPA, Research Triangle Park, NC.*

This presentation discusses methods used to extrapolate from *in vitro* high-throughput screening (HTS) toxicity data for an endocrine pathway to *in vivo* for early life stages in humans, and the use of a life stage PBPK model to address rapidly changing physiological parameters. Adverse outcome pathways (AOPs), in this case endocrine disruption during development, provide a biologically-based framework for linking molecular initiating events triggered by chemical exposures to key events leading to adverse outcomes. The application of AOPs to human health risk assessment requires extrapolation of *in vitro* HTS toxicity data to *in vivo* exposures (IVIVE) in humans, which can be achieved through the use of a PBPK/PD model. Exposure scenarios for chemicals in the PBPK/PD model will consider both placental and lactational transfer of chemicals, with a focus on age dependent dosimetry during fetal development and after birth for a nursing infant. This talk proposes a universal life-stage computational model that incorporates changing physiological parameters to link environmental exposures to *in vitro* levels of HTS assays related to a developmental toxicological AOP for vascular disruption. *In vitro* toxicity endpoints discussed are based on two mechanisms: 1) Fetal vascular disruption, and 2) Neurodevelopmental toxicity induced by altering thyroid hormone levels in neonates via inhibition of thyroperoxidase in the thyroid gland. Application of our Life-stage computational framework to conduct IVIVE illustrated several critical data gaps such as maternal and fetal systemic and intrinsic clearance rates, placental transport kinetics, and partitioning of chemicals in milk during nursing.

3321 PBPK Modeling and Simulation in Drug Development: A Focus on Pediatrics

A. Edginton. *University of Waterloo, Waterloo, ON, Canada.* Sponsor: S. Felter.

PBPK modeling and simulation has found a place in drug development particularly in the areas of drug-drug interaction (DDI) prediction and pediatrics. The development of pediatric pharmacokinetic and safety and efficacy studies is a mandatory activity during the drug development process in North America and Europe. The promise of biologically-based extrapolation to derive the first-in-children dose has been a driving force in the use of PBPK models and they are now recognized by the US Food & Drug Administration (FDA) as a potentially useful tool in the design of pediatric clinical trials and helping to expedite pediatric drug development. Between the years 2008 and 2013, the US FDA's Office of Clinical Pharmacology received 84 NDA/IND submissions containing PBPK models; 18 (21%) of them were pediatric submissions[1]. Historically, the exposure-response relationship has been assumed to be similar to adults and doses are derived based on differential exposure alone. The workflow for PBPK model development of virtual pediatric populations depends on the stage of drug development and the available data (e.g. candidate in development vs. marketed drug product). Model simulation outcomes such as exposure or pediatric equivalent doses as a function of age or weight are used to plan clinical trials. Examples of PBPK model use in planning pediatric clinical trials will be presented including defining dose and simulating DDI potential as a function of age. The outcomes of pediatric PBPK models are only as good as the inputs that have been used to generate them. Ongoing research on closing systems-specific knowledge gaps that will improve our confidence in model outputs will be discussed such as the age dependence of gastrointestinal solubility and small intestinal water volume. Quantifying pediatric-specific system parameters is an important area of future research. [1]. Pan Y et al. Application of the FDA PBPK knowledgebase in evaluating model predictability for drug-drug interactions. ASCPT annual meeting 2014. Clin.Pharmacol.Ther. 2014; 95.

3322a "Breaking Bad": Cardiovascular Autophagy Gone Rogue: A Putative Mechanism of Toxicity and a Drug Target in Disease

L. C. Thompson[1] and T. R. Dugas[2]. [1]*ORISE/US EPA, Research Triangle Park, NC and* [2]*Louisiana State University, Baton Rouge, LA.*

Christian de Duve coined the word "autophagy" in 1963 during his studies of the lysosome. In the 1970s autophagy was identified as a toxicological mechanism in acute liver damage caused by dimethylnitrosamine, and in 1983 rubomycin injection was used to generate a model of myocardial insufficiency in rats by impairing autophagic function in the heart. Autophagy is a tightly regulated process within cells that includes 1) molecular marking of damaged or dysfunctional cellular constituents; and 2) degrading/recycling those marked cellular constituents. Essentially autophagy is a waste management system for cells. By cleaning up worn, aged, and damaged cellular constituents, autophagy helps maintain overall cellular health, function, and efficiency. Disruption of autophagy can have adverse effects on cardiovascular function, and impaired autophagy has been implicated in cardiovascular pathologies and toxicity responses to various pharmaceuticals and environmental toxicants. Abnormal autophagic processes have been well documented in cardiovascular disease and are increasingly being targeted for therapeutic approaches. Thus, autophagy in the cardiovascular system is emerging as an important toxicological and therapeutic endpoint. This session will highlight the clinical consequences of impaired autophagy on normal cardiovascular function, discuss emerging evidence linking alterations in autophagy to cardiotoxicity responses to antineoplastic agents, and examine autophagic consequences in vascular endothelial cells following treatment with drugs used to treat HIV. Finally, this

session will highlight evidence that proposes targeting autophagy as a therapeutic strategy in different vascular diseases. Namely, evidence will be presented describing targeted increase in autophagy to improve endothelial cell-mediated diabetic vasculopathy and decrease smooth muscle proliferation in conditions of atherosclerosis and restenosis.

W 3322b Clinical Perspective on Cardiovascular Autophagy

M. S. Willis. *University of North Carolina, Chapel Hill, NC.* Sponsor: L. Thompson.

Autophagy is a basic catabolic mechanism involving the degradation of cellular components via the lysosomal system. Originally described in the cardiovascular system in heart failure, autophagy has been widely described in cardiomyocytes, cardiac fibroblasts, endothelial cells, vascular smooth muscle cells, and macrophages. An optimal amount of autophagic activity appears critical to the maintenance of cardiovascular homeostasis and function. Depending on the disease context, excessive or insufficient amounts of autophagic flux can contribute to the pathogenesis of disease. Pre-clinical studies offer compelling evidence that the regulation of autophagic flux can alter cardiac pathogenesis. For example, inhibiting autophagy in cardiac ischemia attenuates the disease severity; alternatively, inhibiting autophagy in other clinical contexts (cardiac hypertrophy) can worsen disease. Enhancing autophagic flux in amyloidogenic disease similarly is beneficial in experimental desminopathies and may have potential in heart failure. With new drugs available to alter cardiac autophagy, therapies to treat heart failure may offer new options in human cardiovascular disease in the near future.

W 3323 Contributions of Mitophagy in Cumulative Doxorubicin-Induced Cardiomyopathy

K. B. Wallace. *University of Minnesota, Duluth, MN.*

Impaired autophagy of cardiac cell mitochondria (mitophagy) can contribute to cardiomyopathy and could cause cardiac dysfunction during treatment with antineoplastic drugs. Adriamycin (doxorubicin) is a potent, broad-spectrum antineoplastic agent, the clinical utility of which is severely restricted by the cumulative, dose-dependent increased incidence of cardiomyopathy. Different mechanisms have been proposed to account for this cardiac toxicity, one of which is interference with mitochondrial respiration and bioenergetic homeostasis. One aspect of doxorubicin-induced mitochondrial toxicity is persistent effect on mtDNA oxidation, inhibition of oxidative phosphorylation, altered mitochondrial calcium and respiratory capacity, and histopathology, all of which are clinically silent until critical cumulative doses are attained. In this presentation I will describe current evidence suggesting that this persistent mitochondrial deficit reflects, in-part, a treatment-related failure in mitochondrial homeostasis that is manifest both through altered mitochondrial biogenesis and an inability to effectively clear dysfunctional mitochondria through mitophagic pathways.

W 3324 Cycles of Injury and Repair via Mitophagy Associated with Chronic Exposures and Premature Senescence

T. R. Dugas. *Louisiana State University, Baton Rouge, LA.*

Vascular endothelial cell dysfunction can promote cardiovascular complications, like atherosclerosis, and could result from altered mitochondrial autophagy (mitophagy). Nucleoside reverse transcriptase inhibitors (NRTIs) are a major component of combination therapy that significantly decreased mortality and viral load in HIV-infected patients, but has also been associated with endothelial dysfunction. NRTIs are known mitochondrial toxicants, and our prior studies showed that increased mitochondrial reactive oxygen species (ROS) production could be observed within hours after exposure of endothelial cells to NRTI. Moreover, *in vivo* studies demonstrated that mice treated chronically with some NRTIs exhibit endothelial dysfunction, which is prevented by mitochondrial antioxidant enzyme overexpression. Initially perplexing was that in cell models, the initial drug-induced ROS production resolved by 24h. Subsequent studies showed that mitophagy was activated early after the onset of ROS production, thus allowing for the removal of injured mitochondria. To put these findings within the context of exposures that occur clinically, we hypothesized that for chronic exposures or perhaps with aging, mitophagy becomes less efficient, so as to promote a premature senescence of vascular endothelial cells. Follow-up studies demonstrated that mitochondrial toxicity via oxidative stress results in premature endothelial cell senescence. Ongoing studies are aimed at correlating this premature senescence with altered mitophagic responses, and further, testing whether dietary supplementation with mitochondrial antioxidants can reduce the observed premature senescence. Within the context of aging-related disease progression, NRTIs could serve as a useful tool for understanding the role of mitophagy as an important and necessary repair mechanism.

W 3325 Autophagy as a Drug Target in Diabetic Vascular Disease

J. L. Fetterman. *Boston University, Boston, MA.*

Autophagy is a critical multistep pathway that eliminates damaged proteins and organelles from the cell. Emerging evidence suggests that impaired autophagy contributes to the pathogenesis of cardiovascular disease and may represent a novel pharmacological target. Utilizing novel techniques developed within our laboratory, we are able to assess measures of autophagy in freshly isolated endothelial cells collected from human subjects. We hypothesized that impaired autophagy contributes to endothelial dysfunction associated with diabetes in humans. We measured vascular function and autophagy markers in freshly isolated endothelial cells from subjects with diabetes and non-diabetic controls. Diabetes was associated with endothelial dysfunction characterized by lower brachial artery flow-mediated dilation and impaired activation of eNOS by insulin in endothelial cells. Endothelial cells from diabetic patients had higher levels of p62, a protein that accumulates with reduced autophagic flux and blocking the terminal step of autophagy with bafilomycin in cells from diabetics led to a further accumulation of p62, suggesting intact but insufficient levels of autophagy. Measures of autophagy initiation including rapamycin stimulation, beclin 1 levels, and LC3 puncta were not different. Lamp2a, which facilitates the merger of autophagosomes and lysosomes, was higher in cells from diabetic subjects, possibly reflecting a compensatory response to reduced flux. In endothelial cells from non-diabetic controls, inhibiting autophagy with bafilomycin impaired eNOS activation suggesting that intact autophagy promotes nitric oxide signaling. Spermidine treatment up-regulated autophagy and reversed endothelial dysfunction in freshly isolated endothelial cells from diabetic patients. In conclusion, this work suggests that freshly isolated endothelial cells biopsied from human subjects may serve as a novel screening method for potential therapeutic drugs aimed at improving vascular disease. Additionally, we provide evidence that up-regulation of autophagy may be a novel therapeutic target in the treatment of diabetic vascular disease although additional work will be necessary to refine agents to limit off-target effects.

W 3326 Verapamil, Autophagy, and Vascular Smooth Muscle Antiproliferation

D. J. Conklin. *University of Louisville, Louisville, KY.*

Since it was first identified in the 1960s, the therapeutic manipulation of autophagy has been utilized for the treatment of cardiovascular (CVD) and other diseases, such as cancer. Although many clinically-approved drugs used for the treatment of CVD were not previously thought to stimulate autophagy, some drugs recently have been shown to induce autophagy independent of their conventional mechanism of action. Verapamil, for example, is an L-type calcium channel blocker (CCB) that has been used for over 30 years to treat hypertension, angina pectoris and cardiac arrhythmia. Recently we showed that either pure Verapamil stereoisomers or racemic Verapamil alone induces autophagy and is antiproliferative in cultured vascular smooth muscle cells (VSMCs), indicating a pro-autophagic mechanism independent of CCB action. Such a finding is potentially significant because it reveals a heretofore undefined mechanism of action—one that, when better understood, could be targeted for more efficacious anti-atherosclerotic and/or anti-restenosis therapy. Because the effects of Verapamil on VSMC proliferation are stereo-independent, utilization of higher dosages without CCB activity could eliminate the risk of hypotensive crises. Moreover, study of this antiproliferative and autophagic mechanism of action could reveal a novel therapeutic target in proliferating VSMC, much like mammalian target of rapamycin (mTOR) inhibitors that promote the stabilization of atherosclerotic plaques and prevent restenosis as mediated by drug-eluting stents (DES).

3327 *In Vitro* Dosimetry of Engineered Nanomaterials: Too Complicated to Consider, Too Important to Ignore

S. Hussain[1] and P. Demokritou[2]. [1]*US Air Force, WPAFB, OH and* [2]*Harvard University, School of Public Health, Cambridge, MA.*

Because of the potential public health risk arising from exposure to engineered nanomaterials (ENMs) through consumer applications, a thorough evaluation of their safety is essential. Owing to the fast pace of ENM generation, high-throughput *in vitro* methods for safety assessments are sorely needed, but to date have proven unreliable with limited predictive capabilities extending to *in vivo* models. One major contributor to the discrepancies that exist between these models is a failure to reconcile *in vitro* and *in vivo* dosages. Despite growing evidence of the importance of ENM dosimetry for accurate hazard assessments, few *in vitro* studies take it into consideration. This oversight is likely due to a lack of standardized, easy-to-use, and validated methodologies for dispersion preparation, characterization, and *in vitro* dosimetry estimation. This workshop will highlight recent advancements that strengthen ENM dosimetry, including the development of aerosol lung deposition models, generation of pertinent *in vitro* exposure mechanisms, and integrated approaches for calculating and predicting relevant dosages for both *in vitro* and *in vivo* nanotoxicological examinations. It will also highlight several mature, sophisticated computational tools and experimental methods for obtaining dosimetry information *in vivo* and extrapolating those findings to *in vitro* cellular systems with specific examples related to "real world" ENM exposures. In addition to discussing the current state of the art regarding ENM dosimetry, discussions will center on the need and means for future development of this area.

3328 Emerging Tools and Approaches for Bridging the Gap between Exposure and *In Vitro/In Vivo* Dosimetry of Engineered Nanomaterials for Nanosafety Assessment

P. Demokritou. *Harvard University, Cambridge, MA.*

Because of the potential public health risk posed by the rapidly expanding array of engineered nanomaterials (ENMs) currently in use in many consumer products and industrial applications, high-throughput *in vitro* methods for safety assessment are sorely needed, but to date have not proven reliable in predicting toxicological outcomes in animal models. One of the important reasons for this discordance is the failure to reconcile *in vitro* with *in vivo* doses and link them to "real world" ENM exposure levels. Furthermore, despite growing evidence of the importance of dosimetry in *in vitro* hazard ranking, very few studies take it into consideration. This may be primarily due to a lack of standardized, easy to use, and validated tools and methodologies for dispersion preparation, characterization and *in vitro* dosimetry estimation. This talk will present the importance of measuring cellular dosimetry of ENMs and its implications for the field of nanomaterial safety, and highlight several dosimetric methodologies that can be used in nanotoxicology field with specific examples related to "real world" ENM exposures.

3329 *In Vitro* Aerosol Exposure Systems: Challenges in Dosimetry and Strategic Solution

T. Tilly. *Air Force Research Lab, WPAFB, OH.*

In vitro aerosol exposure chambers (AECs) are being developed to offer a more relevant method to study nanomaterials (NMs) toxicity. AECs are favorable compared to typical submerged cell culture exposures because of their physiologically relevant air-liquid interface cell culture and ability to preserve NMs properties as they are exposed to the cell cultures. Aerosol transport in AECs is complex and characterization of the delivered dose of NMs to the cell culture is necessary to determine the toxicity of NMs aerosols. There have been multiple AECs developed with several different methodologies to determine the deposition of NMs to the cellular targets. These efforts have paved the way in this area of science; however deposition has been shown to be inconsistent within many of the AECs making it difficult to draw any scientific conclusions based on the delivered dose. To transition AEC technology to the toxicological community, dosimetry and particle dynamics in these chambers must be well understood. This talk first will compare review the three mathematic and computational models used to determine the deposition in AECs, and the challenges in assessing and controlling NM dosimetry. Then we will discuss the protocols we developed for exposure and analysis of NMs deposited at the air liquid interface of the AEC. In addition, we will present our results that show variability in deposition of NMs (silver (60 nm), titanium dioxide (30-80 nm) in the AEC and some of the challenges to overcome these using some engineering approaches.

3330 Tools, Frameworks, and Approaches for Translation of Nanomaterial Exposure, Doses, and Responses between *In Vitro* and *In Vivo* Systems for Safety Assessment

J. Teeguarden. *Pacific Northwest National Laboratory, Richland, WA.*

The last five years has seen the rapid maturation of the fundamental concepts regarding the physical, chemical and biological features and processes that control the delivery of nanomaterials to cells *in vitro* and *in vivo*. Paralleling is evolution, increasingly sophisticated computational tools and experimental methods for acquiring dosimetry information have continued to emerge. This talk will present the importance of measuring cellular dosimetry of nanoparticles, the implications for the field of nanomaterial safety assessment, highlight several mature approaches for obtaining dosimetry information *in vitro* and extrapolating those findings to *in vivo* systems with specific examples related to human health risk assessment (calculating a Permissible Exposure Limit for iron oxide nanoparticles).

3331 Advanced Computational Modeling for *In Vitro* Nanomaterial Dosimetry

G. Deloid. *Harvard University, Cambridge, MA.*

Accurate and meaningful exposure or dose metrics are a basic yet stubbornly elusive requirement for *in vitro* screening methods to assess potential health risks of engineered nanomaterials (ENMs). To correctly and consistently quantify what cells "see," during an *in vitro* exposure requires an integrated approach that includes standardized preparation and characterization of ENM suspensions in liquid media, accurate determination of agglomerate size and effective density, and predictive modeling of mass transport. Here we will present the development and validation of advanced computational modeling methods for predicting transport of suspended ENMs in such an integrated dosimetry approach. The gold standard three-dimensional computational fluid dynamics (CFD), and a newly-developed fast and robust iterative one-dimensional Distorted Grid (DG) models will be presented. Validation of the numerical tools by quantitative experimental analysis of flash-frozen, cryosectioned columns of ENM suspensions will also be presented. Case studies will also be presented from using the DG model to predict ENM concentration and deposition metrics for a variety of industry-relevant ENMs suspended in cell culture media. Building upon and integrated with previous work in preparation and characterization of ENM culture media suspensions, these advanced numerical models provide practical tools for obtaining accurate dose metrics needed to enable high-throughput screening of engineered nanomaterials.

3332 *In Vitro* Dosimetry Modeling Will Be a Critical Step Toward Efficient Assessment of Engineered Nanomaterials for Environmental Health and Safety Implications

W. K. Boyes. *US EPA, Research Triangle Park, NC.*

The development and application of engineered nanomaterials (ENM) into commercial and consumer products is far outpacing the ability of traditional approaches to evaluate the potential implications for environmental health and safety. This problem recently prompted the National Nanotechnology Initiative (NNI) to issue a grand challenge to the research and development community to develop the ability to "determine the environmental, health, and safety characteristics of a nanomaterial in a month". This presentation will consider a general framework in which to consider potential for ENM release, environmental fate, transformations, exposures and effects. Within this framework, the role of *in vitro* dosimetry modeling will be illustrated experimentally with examples comparing applied, measured and modeled tissue dose levels of silver and titanium dioxide ENM to cells *in vitro*. To meet the NNI grand challenge will be difficult, and likely require a combination of targeted rapid screening approaches and predictive modeling. A key step will be the ability to understand the dose of ENM delivered to cells in test systems, along with the use of other modeling approaches to rapidly estimate both levels of exposure and hazard within bounds of uncertainty acceptable for the applications being considered.

3333a Medical Device Biomaterials: Challenges in Assessing the Toxicity and Biocompatibility of Nanomaterials, Bioabsorbables, and Tissue Scaffolds

N. S. Goud[1] and P. L. Goering[2]. [1]*Boston Scientific Corporation, Spencer, IN and* [2]*CDRH, US FDA, Silver Spring, MD.*

During the last two decades, there has been an increase in the use of medical devices for various diagnostic and treatment conditions in the healthcare sector. To meet this growing demand, more new and novel materials/chemicals involving cutting-edge technologies are being used in developing those devices and advanced biomaterials. The first speaker will begin the discussion on the toxicological impact of such device materials on patient health. Sometimes, while short-term biocompatibility studies utilizing ASTM and ISO 10993 methods may show passing results, the long-term implant studies may fail. Given the complex characteristics of these new medical devices, the host defense system, including inflammation (innate immunity), acquired immunity, and foreign body reactions must be considered in determining biocompatibility (safety) and efficacy (function). Relationship of surface chemistries to reduction or enhancement of adverse response to the activity of macrophages, lymphocytes, and giant cells will be explored. The next talk will focus on how nano particles are being used for localization and treatment of cancer. The role of nanocomposite materials and stem cells in the development of artificial human organs such as trachea, facial organs, coronary grafts, and heart valves will be discussed. The current status on potential cytotoxicity of nanoparticles will be reviewed and how results from testing of ultra-high concentrations (which are in no way related to the actual doses used in clinic) are creating unnecessary alarm in the public. Discrepancies between *in vitro* and *in vivo* results involving nanomaterials will be described along with the need for a unifying protocol for reliable and realistic toxicity studies. The third speaker will provide insights into a fascinating field of 3-D polymer scaffolds for tissue engineering. Experimental data will be presented on the use of stereolithography-based fabrication with polymers (polylactic acid, polypropylene fumarate, and chitosan, etc.) to simulate internal structure of hepatic lobule and liver vasculature for tissue regeneration, wound healing, and personalized medicine. But these technologies have their own unique challenges, such as sterility, presence of contaminants, bioabsorbable polymers, or monomers, and toxicity of photoinitiator compounds. There will be a discussion on how careful optimization of resin formulation can lead to elimination of cytotoxicity without compromising the accuracy and high resolution of fabrication. Next, the focus will be shifted from materials to fully processed and sterilized devices. Examples of how the presence of detergents, metallic ions, or sterilization residues from processing steps during manufacture can result in biocompatibility test failures will be presented. There will be discussion on case studies with anomalous test results involving cytotoxicity, hemocompatibility, and systemic toxicity, and their impact on patient health. Finally, the last speaker from notified body DEKRA will provide an overview of the regulatory approval process for marketing of medical devices in different EU countries and compliance with the Medical Device Directive. The role of European Medicines Agency in the review of devices containing drugs, pharmaceuticals, biologics, or biotechnology products will be presented. This will be followed by a discussion on the recently released guidance by the European Commission's SCENIHR to assess human health risks of medical devices containing nanomaterials. In summary, this workshop will provide an overview of toxicity issues concerning nanomaterials, bioabsorbables, 3-D tissue scaffolds, and synthetic organs, and strategies to overcome them. The role of device chemistry and host defense mechanisms in long-term test failures will be addressed, as well as examples of anomalous biocompatibility test results, with a discussion of their importance in patients' health, and European guidelines for medical device approvals with special emphasis on devices containing nanomaterials.

3333b Biocompatibility and the Foreign Body Reaction to Medical Device Materials

J. M. Anderson. *Case Western Reserve University, Cleveland, OH.*

The use of biomaterials in tissue engineering, regenerative medicine and nano medicine offers new challenges in the determination of safety of the medical devices for ultimate clinical application. The goal of this presentation is to identify biological design criteria that will prove useful in the development of new biomaterials where both short- and long-term biocompatibility issues are addressed to prevent device failures and ultimately improve patient's health. A unique test method was developed where the device material is placed in a stainless steel mesh cage which is implanted in the subcutaneous tissue of rodents. At various time points, the mesh cages are retrieved to study biodegradation of the test article, histopathological analysis and test exudates for immunochemical parameters. Given the complex characteristics of the medical device materials, the host defense system including inflammation (innate immunity), acquired immunity, foreign body reaction, and wound healing responses were considered in the determination of biocompatibility (safety) and efficacy (function). Fundamental and clinical studies in our laboratory have focused on the development of a mechanistic understanding of the effect of biomaterial surface chemistry on the formation and activity of monocytes, macrophages, lymphocytes, cell adhesion, apoptosis etc. Correlative *in vitro* and *in vivo* studies have identified certain surface chemistries that facilitate a reduction or enhancement of the immune response, at material/tissue interfaces. While studies utilizing ASTM and ISO Standards have shown success in short-term biocompatibility assays, some of these biomaterials (polyurethane and polyethylene terephthalate) have failed in the long-term implantation assays mainly due to the induction of inflammatory cytokines (IL-14 and IL-13) and the subsequent foreign body giant cell formation. Also, one of the challenges frequently encountered with implantable medical devices (such as surgical meshes) is the formation of biofilms and microbial colonization on device surfaces. Various strategies will be discussed such as surface modification of the devices with fibroblast, mesenchymal cell coatings or hydrophilic/hydrophobic coatings in reducing biofilm formation and thus improving device biocompatibility.

3334 Nanomaterials—Their Use in Biomedical Applications Including Development of Artificial Organs: Do the Benefits Outweigh the Risk?

A. M. Seifalian. *University College of London, London, United Kingdom.*

In recent years, nanoparticles (NPs) have increasingly found practical applications in different areas of technology, research and medicine. The small particle size coupled to their unique chemical and physical properties is thought to underlie their exploitable biomedical activities. During the last few decades, the speaker's laboratory, was investigating the use of nanoparticles for localisation and treatment of cancer as well as development of human organs. These organs include trachea, facial organs and cardiovascular, including coronary artery bypass graft, stents, transcatheter heart valves made from nanocomposite materials and stem cells. The focus of this presentation is on the potential toxicity of NPs at clinically relevant doses. Mechanisms of cytotoxicity are discussed and the problem of extrapolating knowledge gained from cell-based studies into a human scenario is highlighted. The so-called 'proof-of-principle' approach, whereby ultra-high NP concentrations are used to ensure cytotoxicity, is evaluated on the basis of two considerations; firstly, from a scientific perspective, the concentrations used are in no way related to the actual doses required which, in many instances, discourages further vital investigations. Secondly, these inaccurate results cast doubt on the science of nanomedicine and thus, quite dangerously, encourage unnecessary alarm in the public. In this context, the discrepancies between *in vitro* and *in vivo* results are described along with the need for a unifying protocol for reliable and realistic toxicity reports.

3335 Strategies in Stereolithography-Based Fabrication and Toxicity Evaluation of 3-D Polymer Scaffolds for Tissue Engineering

S. A. Skoog, P.L. Goering, and R.J. Narayan. *US FDA-CDRH, Silver Spring, MD.*

Stereolithography (SL) is a rapid prototyping technique which has become a valuable tool for fabrication of tissue engineering scaffolds. This technology produces complex structures in a layer-by-layer manner using photopolymerization. The versatility in design and precise nature of SL permits fabrication of complex scaffolds with physiologically relevant architectures. Scaffolds with well-defined pore sizes, interconnectivity, and porosities have been fabricated with structural and mechanical properties that mimic native tissue to permit cell ingrowth, vascularization, and nutrient/waste transport. Advancements in resin composition have enabled fabrication of scaffolds with a wide range of mechanical properties and chemistries for both hard and soft tissue engineering applications. Furthermore, fabrication of patient-specific devices aided by medical imaging systems (e.g., MRI, CT) has expanded the potential of SL for personalized medicine. SL-based scaffolds have demonstrated significant potential for tissue engineering and regenerative medicine; however, several challenges are associated with translation into clinical use, including adequate safety assessment. Toxicological evaluation of SL-fabricated scaffolds includes consideration of device sterility, effects of post-processing, potentially toxic residual components, assay interferences, and challenges with assessment of biodegradable constructs. Scaffolds fabricated from biocompatible polymers may result in adverse health responses due to the presence of contaminants, unpolymerized monomers, and/or residual photoinitiators. Our studies have focused on optimization of resin composition to eliminate cytotoxicity while main-

taining accuracy and high resolution of fabrication. Additional challenges are introduced in biocompatibility assessment when using biodegradable polymers, as these materials are constantly evolving. These studies highlight the need for careful evaluation of adverse biological responses associated with SL-based tissue engineering scaffolds.

3336 Biocompatibility of Medical Devices—Anomalies in Test Results: Discussion on Their Relevance to Patient Safety

N. S. Goud. *Boston Scientific Corporation, Spencer, IN.*

Generally biocompatibility testing is done on fully processed, packaged and sterilized device that is manufactured in compliance to Quality system regulations. So when a biocompatibility test fails, it is an indication that a toxic substance could be present in the device. However, those positive test results can also be due to artifacts in the test system or they are specific only to *in vitro* conditions or observed only in rodent models and may not cause any adverse events in human patients. The focus of this presentation is to discuss specific case studies involving the effect of detergents, oils, metallic ions or sterilization residuals etc. on different assays such as cytotoxicity, hemocompatibility, implantation or systemic toxicity. Unlike pharmaceuticals, medical devices contain a variety of materials such as plastics, metals, fabrics, nanomaterials including trace amounts of adhesives and processing agents etc. Therefore, they have to be cleaned thoroughly during the manufacturing process. Sometimes, the excess detergent residues left on the device due to inadequate washing or if the process is not fully validated, may result in failure of the cytotoxicity assay. It is a challenge to test liquid test articles compared to solid devices. Moreover, device surfaces are often coated with hydrophilic agents for ease of insertion in blood vessels or body cavities. When such devices are being analyzed for biocompatibility, the test samples have to be prepared as per the Directions For Use (DFU) attached to the device. Failure to follow those instructions, may lead to anomalous results. Also, sometimes, the leachable chemicals from the device are known to cause precipitation of proteins leading to false negative results such as in the *in vitro* hemolysis assay. Finally, the process of chemical characterization (ISO 10993-18) of the device and its materials plays an important role in addressing the biocompatibility of a product not only during manufacturing and regulatory approval but also in post market surveillance. This is an interactive session where audience participation is encouraged to share examples of unexpected test results observed in their lab with a discussion on risk assessment and patient safety.

3337 Regulatory Approval of Medical Devices in Europe–Recent Guidelines on Nanomaterials

R. Scherer. *DEKRA Certification B.V., Concord, CA.* Sponsor: N. Goud.

The session will focus on the regulatory approval process for medical devices in the European Union with a specific focus on the Medical Device Directive (MDD). The contents of a technical file/design dossier, conformity assessment routes, and the process for obtaining CE marking for products conforming to these guidelines will be detailed. For granting approval of devices containing drugs, pharmaceuticals, biologics or biotechnology products, the notified bodies (including DEKRA) seek the assistance from European Medicines Agency (EMA). Furthermore, an independent consortium of scientists and physicians derived from universities, teaching hospitals and research institutes are utilized when additional subject matter expertise is required to complete device reviews. The rapid expansion of nanotechnology research within the last decade has led to an increase in novel medical treatment applications in both the pharmaceutical and medical device sectors. With the increase in the level of nanomaterial based medical device submissions, the second half of this talk will focus on the recently released guidance by the European Commission's SCENIHR to assess human health risks of medical devices containing nanomaterials. As there is still much uncertainty in how nanotechnology use in medical devices should be regulated, the speaker will detail the latest developments in nanotechnology regulation with respect to the revised draft MDR and summarize the DEKRA Certification approach to review of these devices, incorporating regulations and standards, device specific assessments, production process evaluations, and nanomaterial based assessments.

3338 Hexavalent Chromium (Cr(VI)) Down-Regulates Acetylation of Histone H4 at Lysine 16 Through Induction of Stressor Protein Nupr1

D. Chen, X. Zhang, L. Fang, T. Kluz, H. Sun, C. Jin and M. Costa. *Environmental Medicine, NYU School of Medicine, Tuxedo, NY.*

The environmental and occupational carcinogen Hexavalent Chromium (Cr(VI)) has been shown to cause lung cancer in humans when inhaled. In spite of a considerable research effort, the mechanisms of Cr(VI)-induced carcinogenesis remain largely unknown. Nupr1 (nuclear protein 1) is a small, highly basic, and unfolded protein with molecular weight of 8,800 daltons. Nupr1 is induced by a variety of stressors such as carbon tetrachloride, starvation, cell cycle arrest, change of media, TGF-beta, and TNF-alpha. Studies in animal models have suggested that Nupr1 is a key factor in the development of lung and pancreatic cancers, with little known about the underlying molecular mechanisms. Here we report that the level of Nupr1 is significantly increased in human bronchial epithelial BEAS2B cells following exposure to Cr(VI). Interestingly, Cr(VI) exposure also results in the loss of acetylation at histone H4K16, which is a common hallmark of human cancer. Cr(VI)-induced reduction of H4K16 acetylation appears to be caused by the induction of Nupr1, since Western blot analysis shows that overexpression of Nupr1 decreases the levels of both H4K16 acetylation and the histone acetyltransferase Mof (male absent on the first; also known as Kat8, Myst 1), which specifically acetylates H4K16. The importance of Nupr1 in the Cr(VI)-induced loss of H4K16 acetylation is further supported by the fact that there is no loss of acetylation of H4K16 upon Cr(VI) exposure in cells expressing small hairpin RNA for Nupr1. Moreover, chromatin immunoprecipitation (ChIP) assays demonstrate that Nupr1-induced reduction of H4K16 acetylation correlates with the transcriptional down-regulation at several genomic loci. We hypothesize that Cr(VI) induces Nupr1 via cellular stress and rapidly perturbs gene expression by downregulating H4K16 acetylation, thereby contributing to Cr(VI)-induced carcinogenesis.

3339 Role of Exosomes Released by Arsenic Transformed Prostate Epithelial Cells in the Recruitment of Stem Cells into an Oncogenic Phenotype

N. O. Ngalame, E. U. Rivera, N. Makia and E. J. Tokar. *NTP, NIEHS, Durham, NC.*

Cancer stem cells (CSCs) drive tumorigenesis, and like normal stem cells (SCs), are affected by their microenvironment. Inorganic arsenic, a human carcinogen, can malignantly transform the human prostate epithelial cell line, RWPE-1 into a cancer phenotype designated CAsE-PE. The human prostate SC line, WPE-stem, can be recruited into CSC-like cells by nearby CAsE-PE cells through unclear mechanisms. Exosomes are small extracellular secreted vesicles that contain biological molecules. Exosomes have been implicated in carcinogenesis as one of the key mechanisms of intercellular communication in the tumor microenvironment. In this study, we profiled the cargo molecules (miRNA, mRNA and proteins) in exosomes released by CAsE-PE cells, and investigated their potential role in regulating the tumor microenvironment during malignant transformation of nearby SCs. Secreted exosomes were isolated from CAsE-PE or RWPE-1 (control) cells. Compared to control cells, CAsE-PE cells secreted 636% more exosomes and CAsE-PE-derived exosomes contained 420% more total RNA. CAsE-PE exosomes differentially expressed (>1.5 fold) 60 out of 84 miRNAs and showed higher levels (mRNA or protein) of oncogenes (e.g. KRAS), inflammatory cytokines, and anti-apoptotic genes compared to control exosomes. To test the role of specific exosome cargo molecules in SC recruitment, we co-cultured CAsE-PE KRAS knockdown (KD) cells or their sham controls with WPE-stem cells. KD of KRAS in CAsE-PE led to KD of KRAS in the released exosomes. Compared with WPE-stem cells co-cultured with sham control cells, WPE-stem cells co-cultured with CAsE-PE KRAS KD cells showed reduced cancer characteristics such as decreased metalloproteinase activity and anchorage-independent growth. These data suggest that arsenic impacts the tumor microenvironment by increasing 1) the secretion of exosomes by the cells and 2) the packaging of select cancer-associated cargo molecules (i.e. KRAS) that play a key role in recruiting SCs into a CSC-like phenotype.

3340 Differential MiRNA and mRNA Expression in Immortalized Human Keratinocytes (HaCaT) After Low Arsenic Exposure Suggest Changes in Cell Proliferation, Cell Migration, Cytoskeleton Remodeling and Carcinogenesis Pathways

L. Al-Eryani[5], S. Jenkins[5], S. Waigel[5], V. A. States[5], V. Arumugam[5], S. N. Rai[5], S. Galandiuk[5], A. K. Giri[1] and J. C. States[5]. *[1]Indian Institute of Chemical Biology, Kolkata, India; [2]Bioinformatics & Biostatistics, University of Louisville, Louisville, KY; [3]Medicine, University of Louisville, Louisville, KY; [4]Pharmacology & Toxicology, University of Louisville, Louisville, KY and [5]Surgery, University of Louisville, Louisville, KY.*

Background: Arsenic is a toxic natural element and widely distributed in the environment. Epidemiological studies have linked chronic arsenic ingestion to several cancers, especially skin cancer. Arsenic-induced skin cancer mechanisms are not well defined, but several studies indicate that mutation is not the driving force as it is for UV-induced skin cancers. Environmentally-driven epigenetic alterations including microRNA dysregulation influence disease development. Preliminary data showed changes in miRNA expression profiles of arsenic induced premalignant hyperkeratosis and squamous cell carcinomas suggesting miRNA dysregulation in malignant progression of arsenic-induced skin lesions. Chronic exposure to low levels of arsenite also malignantly transforms immortalized human keratinocytes (HaCaT). Hypothesis: Differential expression of miRNAs at early stages of arsenite exposure in HaCaT will reveal early changes in the transformation process. Methods: HaCaT were maintained with 0 or 100 nM sodium arsenite for 3 and 7 weeks. Total RNA was purified and miRNA and mRNA expression was assayed using Affymetrix microarrays. Targets of differentially expressed miRNAs were overlapped with the mRNA expression data using Partek Genomic Suite software then mapped to their pathways using MetaCore software. Results: Several miRNAs and mRNAs, including those encoding oncogenes and tumor suppressors, were differentially expressed at 3 and 7 weeks. The data suggest that genes involved in cell proliferation, cell migration, cytoskeleton remodeling and carcinogenesis pathways were induced at 7 weeks. Conclusions: Our data provide strong evidence of early changes in miRNA profiles and their target genes in human keratinocytes contributing to arsenic-induced carcinogenesis. Supported in part by NIH grant R21ES 023627.

3341 Mitochondrial Function Links Non-Linear Dose Response of Arsenite to Growth and Survival in Human and Mouse Primary Hepatocytes

H. D. Chavan[3], K. Mickey[3], P. Christudoss[1], J. Lu[3], R. Swerdlow[3] and P. Krishnamurthy[3]. *[1]Department of Clinical Biochemistry, Christian Medical College, Tamil Nadu, India; [2]Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS and [3]Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Inorganic arsenic is a naturally occurring metalloid that is ubiquitously present in the environment. Epidemiological studies have shown that chronic exposure of humans to inorganic arsenic is associated with liver injury, peripheral neuropathy, and an increased incidence of cancer of the skin, lung, liver, and bladder. However, there has been much controversy about the shape of the arsenic response curve, particularly at low doses. Recent epidemiological and experimental studies have shown that low dose arsenite reduces the relative risk for cancer among exposed populations and extends life-span in metazoans whereas higher concentrations increase toxicity and reduce longevity. Such biphasic response to a potentially harmful compound, commonly termed hormesis, is thought to have a significant impact on growth and longevity. In the present study we found that treatment of human and mouse primary hepatocytes with 0.01 to 100 μM arsenite produces a low dose growth promoting effect while higher dose produces cytotoxic effects. Measurement of mitochondrial bioenergetics shows that arsenite potentiates mitochondrial function at low-dose and inhibits mitochondrial function at high-dose in a manner that correlates well with the expression and activity of mitochondrial respiratory complexes. More importantly co-exposure of hepatocytes to arsenite along with ROS scavengers was able to revert the phenotype of high dose arsenite but not low dose arsenite. Taken together these results suggest a nonlinear dose-response characteristic of arsenite in primary hepatocytes with low-dose arsenite promoting transient increase in mitochondrial function that act as transducers of arsenite induced hormesis.

3342 Cytoskeleton Remodeling and Epithelial-Mesenchymal Transition in Breast Cancer Cells Upon Treatment with Cadmium

Z. Wei and Z. A. Shaikh. *Biomedical & Pharmaceutical Sciences, University of Rhode Island, Kingston, RI.*

Cadmium (Cd) is a carcinogen and well-established human health risk. Studies based on biopsy samples from breast cancer patients have shown greater Cd accumulation in malignant breast tissue than in benign tumor or normal breast tissue. Malignant breast cancer has high migration tendency which is related to cytoskeleton assembly and active cell adhesion site formation. The study described here investigated the effect of Cd treatment on these processes using noncancerous MCF10A cells and metastatic cancer-derived MDA-MBA-231 cells. In MCF10A cells, 1 and 3 μM Cd treatment for 1 h caused integrin β1 activation. Cells treated with 3 μM Cd for 4 weeks had increased integrin β1 and N-cadherin expression, but reduced E-cadherin expression. The changes in these epithelial-mesenchymal transition (EMT) markers indicated that Cd transformed MCF10A cells from epithelial-like to mesenchymal-like. Cells undergoing EMT gain migratory and invasive properties which indicate development of malignancy. In metastatic MDA-MB-231 cells, treatment of cells with 1 and 3 μM Cd for 1-3 h resulted in increased cell spreading and α-actinin staining in filopodia and lamellipodia, indicating more active cytoskeleton formation. Co-localization of active integrin β1 and α-actinin staining indicated that the integrins were involved in the Cd-induced cell spreading process. Western blot results showed that short-term Cd treatment not only activated integrin β1, but also focal adhesion site kinases such as FAK, Src, and ERK1/2. The increased expression of scaffold protein paxillin at focal adhesion site suggested that Cd altered cell motility through integrin-focal adhesion regulation. Treatment of cells with 3 μM Cd for 4 weeks caused an increase in the expression of both integrin β1 and paxillin, and secretion of MMP2/9. It is concluded that treatment of breast cells with low concentration of Cd drives cytoskeletal remodeling through activation of integrin and downstream focal adhesion signaling. Long-term Cd treatment induces EMT transition and may enhance malignancy.

3343 Impaired Glucose Homeostasis in WT and As3MT-KO Mice: Effects of Sex and Arsenic Exposure

C. Douillet[3], M. Huang[3], J. Galanko[3], E. Dover[3], C. Zhang[3] and M. Styblo[3]. *[1]Curriculum in Toxicology, UNC Chapel Hill, Chapel Hill, NC; [2]Medicine, UNC Chapel Hill, Chapel Hill, NC and [3]Nutrition, UNC Chapel Hill, Chapel Hill, NC.*

Chronic exposure to inorganic (i) arsenic (As) has been linked to diabetes in both mice and humans. We have shown that methylated metabolites of iAs formed in the reactions catalyzed by As-methyltransferase (AS3MT) may significantly contribute to the diabetogenic effects of iAs exposure by either inhibiting insulin secretion or insulin signaling. The goal of the present study was to examine glucose homeostasis in wild-type (WT) mice that readily produce these metabolites, and in As3mt-KO mice that cannot efficiently methylate iAs. Male and female mice were exposed to 0, 100 ppb, or 1 ppm As in drinking water. Fasting blood glucose (FBG), area under the curve (AUC) recorded during i.p. glucose tolerance test, fasting and 15-min plasma insulin (FPI, 15PI), and body composition were measured at selected time points. At week 1, FBG and AUC were significantly higher in male KO as compared to male WT mice. Exposures to 100 ppb and 1ppm As increased FBG, but not AUC, in both KO and WT males. In contrast, FBG and AUC were lower in female KO as compared to female WT mice, and little effects of iAs exposure were found. Little or no differences in either FBG or AUC were found at week 8. However, after 12 weeks, male KO mice had 4-6 fold higher FPI and 15PI than male WT mice. In addition, KO males exposed to100 ppb As had higher FPI and 15PI than males in either 0 or 1 ppm group. Similar but less pronounced differences in FPI and 15PI were also observed between WT and KO females. Notably, both male and female KO mice had significantly higher % body fat (27-37%) than their WT counterparts (10-17%).These results suggest that, depending on the exposure time, male mice exposed to low iAs concentrations in drinking water developed impaired FBG, glucose intolerance, and hyperinsulinemia. These effects were more pronounced in KO males that were more obese than WT males. However, KO females that were as obese as KO males were less susceptible to the diabetogenic effects of iAs exposure and obesity. The association between As3mt KO and adipocity in both male and female mice (independent of iAs exposure) warrants further investigation.

3344 Arsenic-Enhanced Atherosclerosis Is Dependent Upon As3MT Expression in Multiple Cell Types

L. F. Negro Silva[1], M. Lemaire[1], D. Plourde[1], V. Slavkovich[2], J. H. Graziano[2], C. A. Lemarié[1], S. Lehoux[1] and K. K. Mann[1]. [1]*McGill University, Montreal, QC, Canada and* [2]*Mailman School of Public Health, Columbia University, New York, NY.*

Arsenic exposure is linked to increased atherosclerosis, in humans and mouse models. Arsenic is metabolized through a series of methylation and oxidation/reduction reactions catalyzed by the arsenic (III) methyltransferase (As3MT) enzyme. However, the role of arsenic methylation via As3MT in the pro-atherogenic effects of arsenic has not been described. We hypothesized that arsenic methylation is important to promote atherosclerosis. We first exposed male apoE-/- mice to 200 ppb methylated arsenicals, and found enhanced plaque formation. Methylated arsenicals also altered the plaque constituents in a manner analogous to our observations with NaAsO2. Furthermore, we compared male apoE-/- and apoE-/-/As3MT-/- (DKO) mice exposed to tap water or 200 ppb NaAsO2 or methylated arsenicals for 13 weeks, and analysed plaque size. Surprisingly, As3MT deletion prevented arsenic-enhanced plaque formation. Arsenic also did not alter the plaque constituents in the DKO mice, as we observed in the apoE-/-. Increased reactive oxygen species (ROS) is a well-reported consequence of arsenic exposure. All apoE-/- groups exposed to arsenicals had significantly increased of ROS staining when compared to control. Remarkably, neither inorganic nor methylated arsenicals induced ROS when As3MT was deleted. Moreover, we have previously described that macrophages are targets for arsenic effects and this cell expresses As3MT. Thus, we investigated the relative contribution of bone marrow (BM)-derived As3MT versus non-hematopoietic-derived As3MT in arsenic-induced atherosclerosis. We carried out BM transplantations where apoE-/- or DKO male mice were irradiated and reconstituted with either apoE-/- or DKO BM. After, mice were given either tap water or 200 ppb NaAsO2 for 13 weeks. ApoE-/- transplanted with apoE-/- BM and exposed to 200 ppb arsenic showed increased plaque formation when compared to controls. Interestingly, neither apoE-/- transplanted with DKO BM nor the DKO reconstituted with apoE-/- BM had increased lesion size after arsenic exposure compared to their respective controls. Collectively, the data revealed that arsenic methylation is necessary for arsenic-increased ROS production in the vessel and that As3MT expression in multiple cell types is mandatory for arsenic-induced atherosclerosis.

3345 Altered Hepatic Transport by Fetal Arsenite Exposure in Diet-Induced Fatty Liver Disease

E. J. Ditzel[2], H. Li[1], C. E. Foy[3], A. B. Perrera[1], P. Parker[1], B. J. Renquist[3], N. J. Cherrington[1] and T. D. Camenisch[1]. [1]*Pharmacology and Toxicology, The University of Arizona College of Pharmacy, Tucson, AZ;* [2]*Regulatory Affairs, Ecolab, Eagan, MN and* [3]*School of Animal and Comparative Biomedical Sciences, The University of Arizona, Tucson, AZ.*

Non-alcoholic fatty liver disease is the most common chronic liver ailment in the United States. It can result in changes to drug metabolism and disposition which leads to adverse drug reactions. It has been established that developmental exposure to arsenite compounds the severity of diet-induced fatty liver disease. This study aimed to examine effects of arsenite potentiated diet-induced fatty liver disease on hepatic transport. Hepatic transporter gene expression changes were detected for Mrp2/3/4 and Oatp1a4/2b1/1b2. Cellular localization for the canalicular efflux transporter Mrp2 was disrupted by arsenite exposure, and plasma concentrations of Mrp substrates were increased. This suggests decreased canalicular export via Mrp2 and increased plasma export via sinusoidal efflux transporters Mrp3/4. In addition, embryonic hepatocytes and adult primary hepatocytes show significantly altered transporter expression after exposure to arsenite in both the presence and absence of Intralipid, a free fatty acid mixture which causes steatosis in primary hepatocytes. This suggests that alterations to hepatic transport as a result of arsenite exposure could manifest early and possibly independently of identifiable fatty liver disease. These data indicate that developmental exposure to arsenite leads to changes in hepatic transport which in turn increases the risk for adverse drug reactions during diet-induced fatty liver disease.

3346 Toxicology Mentoring and Skills Development Training Program

W. K. Rumbeiha[1], A. Correia[1], D. Alexander[2], E. Gilbreath[2], P. Leigh[1] and J. D. Wolt[1]. [1]*Iowa State University, Ames, IA and* [2]*Tuskegee University, Tuskegee, AL.*

A major challenge facing the toxicology profession is a lack of a diversified workforce prepared to address the needs of socially disadvantaged groups who may experience higher exposure and differing response to environmental toxicants than the population at large. Despite concerted efforts, the number of underrepresented minorities entering the toxicology profession remains disappointingly low. One shortcoming is in recruiting undergraduates into toxicology graduate research programs. Toxicology is not commonly taught in undergraduate programs, and therefore, there is widespread unfamiliarity among undergraduates of toxicology as a career path. Iowa State University and Tuskegee University are launching the toxicology mentoring and skills development training program targeting undergraduate underrepresented minorities from historically Black, Hispanic, and Native American undergraduate institutions in order to increase the pool of minorities entering the graduate school pipeline and ultimately the toxicology workforce. A concentrated 1-year program combining mentoring and training opportunities will be used to introduce promising undergraduate STEM majors to toxicology as a career path, with special emphasis on government and industry as the dominant employers of toxicologists. Students and mentors meeting face-to-face in campus and workplace settings will together explore fundamentals of toxicology and the breadth of toxicology and career opportunities. A web-based toxicology training resource will be available to mentors, students and the public-at-large. Because course content will be available to the public, the program will impact an audience beyond that of the mentoring program itself. Mentor-mentee experiences from program participation will be used to gauge the potential and direction for the program as a means to close the gap in diversification of the toxicology workforce by enlarging the pool of well-qualified minority applicants into toxicology graduate programs.

3347 Evaluating Self-Efficacy, Sense of Community, Expectancies, and Science Identity to Increase STEM Persistence in Undergraduate STEM Students

R. S. Pollenz and D. Findley-Van Nostrand. *Office for Undergraduate Research, University of South Florida, Tampa, FL; Psychology, University of South Florida, Tampa, FL.*

Persistence of undergraduate students in STEM disciplines is an issue of national concern. This issue is particularly relevant to the field of toxicology because the number of BS-level toxicology programs is limited and undergraduate matriculation to graduate level toxicology programs occurs from many STEM majors. In addition, the ability to inspire undergraduate students toward the field of toxicology at institutions that do not offer a BS level toxicology degree typically occurs through upper level toxicology-based elective courses that students who leave the STEM may never have a chance to take. USF offers several 5.5. day STEM Academy programs to inspire students to STEM undergraduate degree programs. These programs are hypothesized to build community, increased self-efficacy, and science identity within the student cohorts, and these cognitive changes are expected to increase engagement and persistence in STEM. Thus, we have assessed these constructs via survey measures adopted from existing instruments. The survey was piloted in a population of STEM/non STEM majors and in a 5.5 day high school program. In the pilot study, STEM majors scored higher than non-STEM majors in: self-efficacy for STEM-coursework, scientific identity, feelings of belongingness to STEM overall, positive motivation for STEM career, and task goal orientation. The data also show that STEM identity, sense of belonging/community to STEM and to the university, and positive expectancies regarding a STEM career are negatively correlated with intention to leave STEM. In the high school program, paired t-tests were conducted to examine mean-level changes between the pre and post results. The results show significant post-survey mean-level increases ($p<0.05$) in: self-efficacy for science-related tasks, self-efficacy for STEM-coursework, scientific identity, family support of STEM career, positive motivation for STEM career, and task goal orientation. Collectively, these results confirm that a short, cost effective 5.5 day engagement program can increase sense of community to the university and STEM discipline, and may also impact other cognitive mechanisms. Longitudinal analysis is underway with current college-age cohorts to determine the impact on STEM persistence. Portions of this study were supported by institutional grant 52008123 from HHMI.

3348 Intensive Research Training Improves Undergraduate Student Scientific Competencies During a 10-Week Summer Fellowship

S. M. Marco[4], L. E. Liang[2], R. Riley[1], K. Nakata[1], D. L. Laskin[4] and L. M. Aleksunes[4]. [1]*Center for Environmental Exposure and Disease, Piscataway, NJ;* [2]*Center for School and Community-Based Research and Education, Piscataway, NJ;* [3]*Dept of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ and* [4]*Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ;* [5]*Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

The Summer Undergraduate Research Fellowship (SURF) program at Rutgers University promotes pharmacology, toxicology and environmental health sciences as careers in biomedical research. The program consists of 10-week research training experiences for undergraduates and weekly activities including laboratory safety and responsible conduct of research training, a field trip to a pharmaceutical company, career development, research seminars, and student presentations. To assess participant outcomes from SURF, students completed an adapted version of the Undergraduate Research Student Self-Assessment survey on weeks 1, 5, and 10 of the 2014 and 2015 programs. The response rates for the pre-, mid-, and post-program surveys were 100%, 96%, and 94%, respectively. The surveys identified changes in three key areas over the course of the fellowship: Application of Knowledge to Research (e.g. data analysis and problem solving), Scientific Abilities (e.g. daily independent and group activities), and Research Abilities (e.g. laboratory and presentation activities). Participants used a 7-point scale to assess their level of confidence on 27 competencies. Significant improvement was observed in all competencies from pre- to post-program. The students reported the greatest improvement in 3 areas: ability to determine the next step in a research project (29%), write scientific reports or papers (28%), and give oral presentations (28%). A summer research program engages undergraduate students in full-time research and provides unique opportunities to promote toxicology and environmental health sciences as research careers for the next generation of scientists. Supported by the SOT Intern Program, ASPET SURF, P30ES005022 and R25ES020721.

3349 Use of Biological Pathway Databases, Primarily Open Source

M. E. Gillespie. *PHS, St. John's University, Jamaica, NY.*

Sifting through the results of expression or other data rich analyses posses a daunting challenge to experienced and novel researchers alike. Numerous methods exist, all sharing the common theme of grouping the results in such a way as to assist the researcher in finding the underlying patterns in the data. Biological pathway knowledgebases provide a platform for the identification of pathways that are represented within the experimental data. Reactome is an open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatic databases. Pathway and Expression Analysis tools analyze user supplied datasets permitting ID mapping, pathway assignment and over-representation analysis. The rich detail of the Reactome datset combined with accessible analysis tools present toxicologists with a easy to use computational pipeline for biological pathway analysis. Identifying the relevant toxicological pathway from molecular participants is a daunting task. Whether the researcher is taking a detailed look at a small number of proteins or sifting through a large data set identify the functional biological pathway is a critical step. Reactome is an open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatic databases. Pathway and Expression Analysis tools analyze user supplied datasets permitting ID mapping, pathway assignment and over-representation analysis. The rich detail of the Reactome datset combined with the analysis tools

3350 From the High School Classroom to the Laboratory Bench: Design of a One-Week Toxicology Summer Program

G. L. Guo, A. Venosa, J. Szilagyi, S. M. Marco, L. M. Aleksunes, A. J. Gow and D. L. Laskin. *Dept. of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

In recent years, graduate programs have developed platforms to introduce undergraduate students to careers in toxicology. However, little curricula have been developed to increase the literacy of high school students about our discipline. The Toxicology, Health and Environmental Disease (THED) Summer Program provides high school students with lecture, discussion and lab-based activities over a 1-week period to increase their exposure to the biomedical sciences and toxicology. Pre- and post-surveys are used to assess student motivation for participation and understanding of course topics. Over the first 3 years of the THED program, 144 students entering 10th-12th grade have participated. The three most frequent reasons for students to enroll in the program include 1) gaining hands-on laboratory experience, 2) exploring their interest in science and 3) clarifying a college major. Self-assessment of research abilities for all students revealed improved confidence in DNA isolation (42%), analysis of patient findings (31%), cell work and microscopy techniques (28%) and oral exposition (20%). Interestingly, whereas gender analysis did not reveal significant differences, grade level showed rising sophomores improved team work (22%) compared to an overall 9% improvement across all grades. A true/false quiz tested student understanding of general concepts in toxicology, histopathology, dose response and genetics. The average score increased from 66% in the pre-survey to 82% in the post-survey. Further analysis revealed greatest improvement in the areas of histopathology and dose-response relationships. In addition, upperclassmen reported greater satisfaction with laboratory-based activities, while more junior participants were interested in meeting other students. In conclusion, the THED Summer Program successfully enhances the literacy of high school students about the discipline of toxicology and provides them with unique laboratory and career development training. Supported by P30ES005022, AR055073 and T32ES007148.

3351 Technology Transfer Enabling a Participatory Research Project of a High School Community to Measure Organisms Involved in Dioxin Degradation and Antibiotic Resistance

B. L. Upham[4], M. Fitschen-Brown[4], R. Stedtfeld[4], G. Zylstra[3] and S. Hashsham[4]. [1]*Civil and Environmental Engineering, Michigan State University, East Lansing, MI;* [2]*Civil and Environmental Engineering and Center for Microbial Ecology, Michigan State University, East Lansing, MI;* [3]*Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ and* [4]*Pediatrics & Human Development, Michigan State University, East Lansing, MI.*

The Superfund Research Program at Michigan State University is working with high school students to translate molecular biology tools to assess genes related to specific functions. Traditional molecular biology approaches are complex and require expensive instrumentation. Therefore they and carried out in a centralized well-equipped lab by trained personnel. Recent advances in molecular biology protocols, device development, and simple microfluidic systems have made it possible to carry out many (but not all) molecular biology measurements under field conditions. Such developments include elimination of DNA extraction when using isothermal approaches, use of hand-held gene analyzers (e.g., Gene-Z), and self-directed liquid dispensing systems. High school students are being recruited to examine genetic markers in river-water samples. A number of functional genetic targets are being studied in parallel including antibiotic resistance genes, mobile genetic elements (intI1), and functional genes related to dioxin degradation (bph). Complementing the Gene-Z device, loop mediated isothermal amplification (LAMP), which is less influenced by inhibitors compared to PCR is being used for molecular amplification without DNA extraction/purification. Assay validation includes testing both type strains and surface water samples spiked with type strains, and testing via LAMP on the Gene-Z and qPCR on a conventional thermal-cycler. Validated assays and Gene-Z devices are being provided to high-school students for field based testing of water and sediment along the Tittabawassee River in Midland, Michigan. Taken together, these tools provide high school students an opportunity to learn basic principles of: i) molecular biology and genetic markers including primer design and experimental measurements, ii) simple microfluidics and chip fabrication, iii) demonstrate the use of genetic approaches to characterize anthropogenic pollution, and iv) learn how scientists and government agencies use science to address such issues.

3352 Toxic Beauty: Campus-Community Education and Outreach on Cosmetics and Personal Care Products

T. Dodd-Butera[1] and M. Broderick Pritty[2]. [1]*CSU San Bernardino, San Bernardino, CA and* [2]*San Diego Division, California Poison Control System, San Diego, CA.*

Cosmetics and personal care products have been listed in the top 5 substance classes involved in human exposures, as cited by the Poison Control System annual report from the National Poison Data System (2013). The majority of these acute exposures involved minor or non-toxic outcomes. However, toxic exposures have been documented from heavy metal exposures and adulterants. A local radio reporter reached out to a campus faculty member for an educational opportunity to engage in questions and answers regarding the toxicity of beauty products. The discussion was then taken back to share with students and faculty for an interdisciplinary educational campus forum, using a modification of appreciative inquiry (AI) (Hammond, 1998). A focus on the existing Federal Food Drug & Cosmetics Act (FD&C Act, sec. 201(i) for safety and the limits of protection was discussed. Further, the global reach of poisonings from products outside the regulatory guidelines of the USA as a circumstance and source of exposure was reviewed. Topics included mercury in skin lightening products from the Dominican Republic (McKelvey et al., 2011), lead exposure in lipstick, and acute vs. chronic toxicity. This was an educational opportunity with both campus and community input to explore symptoms and sources of acute poisonings and chronic exposures; and propose prevention strategies and a public health perspective on toxicology and cosmetics/personal care products.

3353 A Semester-Long, Inquiry-Based, Lab Using the Aryl Hydrocarbon Receptor (Ahr) in an Upper-Level Undergraduate Biology Course

L. M. Williams. *Biology, Bates College, Lewiston, ME.*

Recommended by Howard Hughes Medical Institute and the National Research Council, amongst others, inquiry-based labs that span the entire semester expose students to a more realistic research situation. With this in mind, a semester-long project for an upper-level undergraduate biology course was developed and carried out in four different semesters from 2013 to 2015 to explore the role of toxicants in regulating the expression of cytochrome P450 genes via the aryl hydrocarbon receptor. Using the zebrafish as a model and working in pairs toward the single goal of determining cis-regulation of cyp1c1 via Ahr2, students cloned the promoter of the cyp1c1 into a plasmid containing eGFP and luciferase as reporter genes. Over the course of the semester, students learned and practiced molecular biology and toxicological techniques including bioinformatics, DNA isolation, PCR, gel electrophoresis, ligation, transformation, plasmid preparation, mammalian cell culture, transfection, chemical dosing, confocal microscopy, and the luciferase assay. Using the primary literature, students chose chemical(s) known to be agonists of the Ahr and determined appropriate dosing plans. At the end of the semester, results were statistically analyzed and verbally presented to the class. Results of pre- and post-semester surveys indicate that student knowledge about experimental design and best practices in the lab increased significantly, student confidence related to research was enhanced, and student interest was held through the entire semester.

3354 "Talksicology": Effective Oral Presentation Techniques

B. L. F. Kaplan[1] and R. Pollenz[2]. [1]*Mississippi State University, Mississippi State, MS and* [2]*University of South Florida, Tampa, FL.*

Effective dissemination of research findings in seminars, interviews, scientific meetings, or to the public has always been a critical skill for toxicologists. While effective oral presentation skills are formally taught in most training programs, gaps in the training exist and many programs do not measure success or offer direct evidence of effectiveness. The main goal of this workshop is to provide attendees with an opportunity to assess effectiveness, and improve their own presentations and oral communication skills. The session is designed to be engaging and interactive. The first part of the workshop will be the analysis of a "flawed" presentation in real time in which the audience will use smart phone-based technology (PollEverywhere) to rate and discuss the presentation. This exercise will be followed by short sessions from three experts from academia and industry who will provide examples and techniques for oral presentations specific to 1) a research seminar, 2) an interview, and 3) a situation in which the scientists must communicate to the media or lay public. Effective oral communication during poster presentations will also be presented. This workshop will be applicable for anyone wishing to enhance oral communication skills and is particularly pertinent to developing scientists who want to improve in this craft.

3355a Mitochondrial Dysfunction as a Pathogenic Mechanism and Therapeutic Target for Neurodegenerative Diseases

K. Tieu[1] and A. Bowman[2]. [1]*Plymouth University, Plymouth, United Kingdom and* [2]*Vanderbilt University, Nashville, TN.*

Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis are characterized by a relatively selective loss of one or a few populations of neurons in specific brain regions. Major advances have been made over the past decades toward understanding mechanisms of neurodegeneration. These insights into how neurons die in disease can now be leveraged to develop effective therapeutic strategies. Mitochondrial dysfunction has emerged as a common feature in neurodegenerative disorders. This observation is consistent with the high vulnerability of mitochondria to environmental toxicants (such as herbicides, pesticides, and heavy metals) as well as disease-linked mutation in genes that are functionally associated to mitochondrial biology. This symposium has brought together six leading scientists from industry and academia to discuss the latest information about how various toxicants impair mitochondrial function contributing to neurodegenerative processes and how these experimental models are used for development of novel therapeutic strategies. Although the focus will be on toxicology, latest information about genetic mutations and gene-environment interactions that impact mitochondrial function will also be incorporated in this session. To emphasize how the diverse biology of mitochondria can contribute to disease, several of the talks are focused on a specific disease, Parkinson's disease (PD), to highlight the emerging understanding that mitochondria dysfunction is multifaceted. However, the mechanisms and therapeutic strategies highlighted in this symposium are relevant to other diseases with mitochondrial etiology. Further, speakers will present on the utility of diverse but complementary experimental models. To kick off the session, Ian Reynolds from Teva Pharmaceuticals will provide an overview of mitochondrial biology in neurological diseases, with focus on mitochondria as a therapeutic target. This high-content introduction will establish a common frame of reference for the remaining five talks. Julie Andersen will discuss a novel mechanism by which a strong genetic risk factor for PD, PARK2 (parkin), influences mitochondrial quality control. To highlight potential links of this genetic risk factor with environmental risk, Aaron Bowman will discuss new data suggesting PARK2 mutations increase sensitivity of human neurons to Cu-induced mitochondrial dysfunction and mitochondrial fragmentation. These data support an involvement of mitochondrial fission and fusion imbalances in disease. The therapeutic potential of targeting mitochondrial fission/fusion machinery in PD will be detailed by Kim Tieu in primarily toxicant-induced mammalian cell culture and mouse models. Manganese (Mn) is an established environmental risk factor for PD. Michael Aschner will report on the protective effects of a novel protein, TMEM-135, against Mn-induced oxidative stress and toxicity in the genetic model organism, *C. elegans*. The final speaker, Dean Jones, will conclude the session with a cutting-edge omics approach to gain a broad and comprehensive picture of mitochondrial regulation and signaling in response to toxic insults. In summary, this session is designed to comprehensively cover the complex nature of mitochondria as a pathogenic pathway and therapeutic target for neurodegenerative disease(s) with environmental etiologies.

3355b Mitochondrial Neurotherapeutics: Past, Present, and Future

I. Reynolds. *Teva Pharmaceuticals, West Chester, PA.* Sponsor: K. Tieu.

Mitochondria in the central nervous system have a critical role in brain injury. CNS mitochondria represent an important potential target for the toxic effects of xenobiotics, and also occupy a central position in the pathogenesis of numerous neurodegenerative diseases. The full range of mitochondrial functions that are impacted in disease states is still being fully elucidated, but include alterations in bioenergetics, mitochondrial trafficking and a range of cell homeostatic processes. Mitochondria may also contribute to the injury of neural cells by the release of reactive oxygen species or apoptogens. The centrality of mitochondria in pathogenesis also suggests that mitochondria may repre-

sent an interesting and novel target for neuroprotective therapeutics. This presentation will provide examples of the roles that mitochondria play in neurodegenerative diseases, review experiences with mitochondrially targeted neuroprotective agents and discuss novel approaches to modulating mitochondrial function for therapeutic purposes.

S 3356 Parkin-Mediated Mitochondrial Quality Control via the Master Lysosomal Regulatory Factor TFEB Is Disrupted in a Mutant Mouse Model of Parkinson's Disease

J. K. Andersen. *Buck Institute for Research in Aging, Novato, CA.*
Sponsor: K. Tieu.

Mutations in the PARK2 gene (parkin) have been reported to result in reductions in mitochondrial turnover, resulting in increased levels of defective mitochondria. This is widely held to be the consequence of losses in parkin E3 ligase activity that normally ubiquitinates damaged mitochondria, marking them for degradation by the lysosome. Here we report that while levels of mitophagy are reduced in a parkin mutant mouse model expressing a C-terminally truncated parkin mutation selectively within DAergic neurons, this appears to involve a late-stage blockage in autophagic-lysosomal pathway (ALP) function. Treatment with the autophagic inducer rapamycin restores mitochondrial turnover and function, as well as the development of associated neurodegenerative features in this model in a manner not requiring restoration of lost E3 ligase function. Rapamycin also prevents reductions in expression of the master mitochondrial regulator peroxisome proliferator-activated receptor-gamma coactivator (PGC1alpha) and its downstream target, the lysosomal transcription factor transcription factor EB (TFEB). We propose that in addition to its known interaction with PTEN-induced putative kinase 1 (PINK1), parkin may also elicit mitochondrial turnover via an alternative pathway involving PGC1alpha-TFEB signaling. This constitutes a novel role for parkin in relation to mitophagy and validates PGC1apha-TFEB as an important therapeutic target for PD.

S 3357 PARK2 Mutations Alter Vulnerability of Human Neurons to Copper Neurotoxicity

A. Bowman. *Vanderbilt University, Nashville, TN.*

Poorly-defined interactions between environmental and genetic risk factors underlie Parkinson's disease (PD) etiology. We tested the hypothesis that human stem cell derived neurons from patients with known familial risk for early onset PD (biallelic PARK2 mutations) will exhibit enhanced sensitivity to PD environmental risk factors compared to healthy control subjects without a family history of PD. We report that PARK2 mutant neuroprogenitors showed increased sensitivity to acute 24-hour cytotoxicity with copper (Cu) and cadmium (Cd) exposure but not manganese (Mn) or methyl mercury (MeHg) relative to control neuroprogenitors. PARK2 mutant neuroprogenitors also showed a substantial increase in mitochondrial fragmentation, initial ROS generation, and loss of mitochondrial membrane potential following acute Cu exposure. These data support Cu exposure as an environmental risk factor for PD/parkinsonism. We also observed a shift in the lowest observable effect level (LOEL) for greater sensitivity to Cu-dependent mitochondrial dysfunction. These data will be presented in the context of other PD genetic and environmental risk factors that link mitochondrial dysfunction and risk for PD.

S 3358 Targeting Mitochondrial Dynamics As a Potential Therapeutic Strategy for Parkinson's Disease?

K. Tieu. *Plymouth University, Plymouth, United Kingdom.*

Mitochondrial dysfunction is a pathogenic mechanism in both familial and sporadic Parkinson's disease (PD). However, effective therapy targeting this pathway is currently inadequate. Mitochondria undergo frequent changes in shape, size, number and location. These alterations are affected by mitochondrial morphology, which in turn, is controlled mainly by mitochondrial fission and fusion proteins. Targeting these proteins to manipulate mitochondrial dynamics (fission, fusion and movement of mitochondria) has emerged as a potential novel therapeutic approach for Parkinson's disease. We report here the potential clinical relevance of this strategy by providing *in vitro* and *in vivo* evidence in both genetic (Pink1-/-) and toxicant-induced (MPTP, rotenone, paraquat) models of PD. Specifically, we show that inhibition of mitochondrial fission by blocking the function of GTPase dynamin-related protein-1 (Drp1) using gene-based and small molecule strategies improves mitochondrial function, attenuates neurotoxicity and synaptic deficits in these models. These results suggest Drp1 inhibition as a potential treatment for PD.

S 3359 Mitochondrial TMEM-135 Decreases Manganese-Induced Dopaminergic Neurodegeneration

M. Aschner. *Albert Einstein College of Medicine, Bronx, NY.*

Environmental exposure to manganese (Mn) is an occupational health hazard that may result in a clinical picture analogous to Parkinson's disease (PD). We recently established that a novel protein, TMEM-135, attenuates the pathology of Mn-induced neurotoxicity. TMEM-135::GFP expressing worms (*C. elegans*) showed increased survival in the cold. The mitochondrial action potential sensing dye, MitoTracker was also used to compare fluorescence in the three *C. elegans* strains. We found a significant reduction in MitoTracker fluorescence in tmem135-/- animals compared with wildtype (WT) controls. Functional loss of TMEM-135 was associated with a reduction of DAF-16 expression (the homolog of human FoxO) compared with WT controls. Furthermore, TMEM-135::GFP overexpression led to a 2-fold up-regulation of DAF-16 levels compared with WT controls. TMEM-135 likely works in cooperation or upstream of DAF-16 to modulate oxidative stress response genes in mammals and in *C. elegans*. Given the conserved mechanisms of dopaminergic (DAergic) cell function between *C. elegans* and humans, and the analogies between manganism and PD, we posit that TMEM-135 with its known role in preservation of fat stores and mitochondrial function affords protection against Mn-induced oxidative stress injury in DAergic neurons.

S 3360 Integrated Redox Proteomics, Metabolomics and Transcriptomics: A Holistic View of Mitochondrial Function

D. Jones. *Emory University, Atlanta, GA.*

This talk will highlight advances in mass spectrometry based redox proteomics and high-resolution metabolomics of mitochondria, used in combination with nuclear transcriptome analysis, to obtain more comprehensive understanding of mitochondrial regulation and signalling in response to environmental and toxicological challenges. Redox proteomics provides measures of specific cysteines in mitochondrial proteins, and metabolomics allows identification of metabolic changes that occur in association with the protein oxidation. Transcriptome changes show downstream responses that occur in association with the protein and metabolic changes. Advanced bioinformatic and visualization methods facilitate rapid and efficient evaluation of the complex data. Specific published and unpublished examples from on-going research will be provided. These include studies of mitochondrial toxins such as rotenone, inhibitors of thioredoxin reductases (auranofin), mitotoxic transition metals (cadmium and manganese), mitotoxic pesticides (maneb and paraquat) and blue light-induced toxicity.

W 3361a Beyond Benchmark Dose: Advancing Probabilistic and Bayesian Approaches in Hazard Characterization

N. B. Beck[1] and A. M. Jarabek[2]. [1]*American Chemistry Council, Washington, DC and* [2]*US EPA, Research Triangle Park, NC.*

The National Academies (NRC) and other expert review bodies have frequently recommended that hazard assessments use Bayesian or probabilistic approaches in evaluating uncertainty and/or variability in hazard characterization. Most recently, in a 2014 review of EPA's Integrated Risk Information System (IRIS) program, the NRC reiterated its suggestion to use Bayesian analyses to inform quantitative judgments about hazard and dose response. In this report, unlike other reports on the IRIS program, the NRC provided specific examples illustrating how such approaches could be incorporated. Similarly, the WHO/IPCS has also provided recent advice on using probabilistic approaches to characterize hazards in lieu of point estimates. In addition, computational approaches are progressing that provide for description of key events in a prognostic fashion that can facilitate the application of advances in systems biology as well as meta-analysis and data integration. Such computational approaches offer the promise of network analyses and leveraging of new data streams in a read-across fashion. This workshop session will explore the recent recommendations provided by both NAS and WHO and will illustrate with case studies how these approaches, as well as other probabilistic and Bayesian approaches, can be implemented to inform hazard assessments.

3361b Setting the Stage: Recent Advice from the National Academies on Bayesian Analysis in Hazard Assessment

Y. Zhu. *University of South Florida, Tampa, FL*. Sponsor: N. Beck.

In 2011 a National Research Council (NRC) committee that reviewed EPA's assessment of formaldehyde made a number of recommendations on how to improve the quality of the IRIS documents. In response to the NRC committee recommendations, EPA made a number of changes to the IRIS process. In May 2014, the NRC provided a review of the progress being made within EPA's Integrated Risk Information System (IRIS). The NRC's review includes further recommendation of using Bayesian methods to strengthen risk characterization. Recognizing that the Bayesian framework is structured for integrating evidence across heterogeneous data systems and for probabilistic quantification of uncertainties of varying degrees across these systems, the call for Bayesian analysis in hazard characterization seems quite appropriate. Bayesian analysis was used as examples in the context of hazard identification to quantify expert judgments using a scale of probabilistic certainty, an approach that has been taken in the Intergovernmental Panel on Climate Change (IPCC) reports. Bayesian approaches were also illustrated in the context of evidence integration to not only help identify gaps in knowledge but to also understand uncertainty of different sources and types. Bayesian hierarchical models were explicitly used to integrate data of cross-species relevance or of mechanistic similarity and to derive overall risk estimates and uncertainties, in particular the derivation of toxicity values. This first talk will walk the audience through some of the specific examples presented by the NRC report and will also discuss the benefits of incorporating Bayesian frameworks into hazard characterization.

3362 Bayesian Methods for Uncertainty Factor (UF) Application: Proof-of-Concept and Pitfalls

T. W. Simon. *Ted Simon LLC, Winston, GA.*

For 24 recent IRIS assessments, UFs were applied using the Bayesian methodology described in NRC's 2014 review of IRIS in which the uncertainty in the point of departure is combined with the overall uncertainty by expressing these as the standard deviations of lognormal distributions. The five areas of uncertainty encoded by the UFs are: human variability, animal-to-human extrapolation, subchronic-to-chronic extrapolation, LOAEL-to-NOAEL extrapolation, and database insufficiency. When the composite UF, the product of all five individual UFs is expressed as a standard deviation and combined with the uncertainty in the POD, as the example in the NRC review, the resulting RfDs are about two-fold less stringent than those using traditional application of UFs. When the individual UFs were each expressed as a standard deviation and combined individually with the uncertainty in the POD, the resulting RfDs are about fifteen-fold less stringent than the traditional methods. The primary focus of the NRC document was the future—providing methods to combine uncertainty from vastly different data streams, for example, animal testing and *in vitro* assays. However, before the Bayesian methods can be applied for ongoing IRIS assessments, combination of UFs and several other issues need to be addressed. The overall purpose of this exercise was to determine the ease of use of the Bayesian methodology, to find unanswered questions, and to begin a dialogue to provide answers.

3363 Probabilistic Dose-Response Assessment: Basic Principles and General Approach Developed by the WHO/IPCS

W. A. Chiu. *Texas A&M University, College Station, TX.*

WHO/IPCS recently published a guidance document on evaluating uncertainties in dose-response assessment/hazard characterization. The recommended approach differs in two ways from more traditional dose-response assessments. First, rather than single values for the point of departure (POD) and for the adjustment/uncertainty factors, it uses uncertainty distributions derived from historical (i.e. "prior," in a Bayesian context) and/or chemical-specific data and analyses on each of those aspects. Second, it quantitatively defines the protection goals in terms of incidence (I) and magnitude (M) of the critical effect in the human population. By contrast, traditional approaches for developing a human exposure limit result in a single value (e.g., RfD, ADI) for which the associated values for I and M are not quantified and the uncertainty in that value remains unknown. In the probabilistic approach described by WHO, the values of I and M are made explicit, while the uncertainty in the target human dose at these values of I and M is quantified. Risk managers can use this additional information to better weigh potential human health effects associated with different risk management options against other considerations within their specific problem formulation context. Further, if they consider the overall uncertainty to be unacceptably large, the probabilistic analyses can inform the value of information associated with different data generation, research, or analysis options for developing a higher tier assessment. This presentation focuses on the basic principles and approach underlying the WHO/IPCS framework.

3364 Leveraging High-Dimensional Data to Inform Probabilistic Dose-Response Estimates

D. M. Reif. *North Carolina State University, Raleigh, NC.*

The emergence of high-dimensional toxicological datasets that cover diverse areas of chemical and biological space can be used to inform estimates of quality, uncertainty, and potency in probabilistic hazard assessment. These new data, generated in a highly systematic manner across partially-related sets of assay endpoints, may provide context for individual dose-response assessments. Global patterns of responses across diverse chemicals and assays are used to help define reasonable bounds for expectation. These globally-estimated bounds are then applied to individual assessments of quality (What is the probability that a given dose-response is real, given data on similar chemicals and assays?), uncertainty (What were the distributions of responses in related experiments?), and potency (How do characteristic activity concentrations compare across test systems?). This contextual approach thus leverages the power of high-dimensional data to provide alternative, empirical evaluation of variability in dose-response assessment.

3365 Consensus Modeling in Support of a Semi-Automated Read-Across Application

R. Judson. *US EPA NCCT, Research Triangle Park, NC.*

Read-across is a widely used technique to help fill data gaps in a risk assessment. With the increasing availability of large amounts of computable *in vitro* and *in vivo* data on chemicals, it should be possible to build a variety of computer models to help guide a risk assessor in doing a read-across assessment. We are developing a suite of "local models" which take available data in all local neighborhoods of chemical space. (The space of chemicals being evaluated covers most of the ~30K for which there is known use.) These are a variety of machine learning and Bayesian models that make probabilistic calls regarding specific endpoints based on availability of data in the local neighborhood. Different models use different descriptor sets, distance metrics and scoring functions. The models are then combined into a consensus, which attempts to minimize or cancel out the bias inherent in each individual model. The performance of the models in each local neighborhood for each endpoint is then evaluated statistically. The models are finally incorporated into a prototype read-across dashboard. This talk will discuss the modeling approach, will walk through case studies and demonstrate the dashboard itself.

3366 Facilitated Discussion on Bayesian Approaches in Hazard Assessment

A. M. Jarabek. *US EPA, ORD, Research Triangle Park, NC.*

The talks in this workshop have shown that advancing probabilistic methods and incorporating Bayesian approaches into hazard characterizations is something that can be done, albeit with differing levels of difficulty, complexity and ease. The workshop session will close with a facilitated discussion that will further discuss how to address challenges, advance the approaches presented, and compare and contrast the methodologies for different needs. Discussions will also focus on the benefits and strengths of the approaches.

3367a Bringing More Science into the Process of Risk Assessment for Endogenous Chemicals with Exogenous Exposures

W. Farland[1] and A. Lynch[2]. [1]*Colorado State University, Fort Collins, CO and* [2]*American Chemistry Council, Washington, DC.*

The endogenous presence in the body of chemicals that also have exogenous exposures poses a challenge to risk assessment. Endogenous is defined as "produced internally in the body," typically as a result of intermediary metabolic function. In current risk assessment practice, there is not a standard approach for addressing exposure to endogenous levels of chemicals or chemicals with non-anthropogenic back-

ground exposures such as those from common, natural dietary sources ("natural background"). Determination of what criteria to consider when evaluating an exogenous chemical with endogenous exposure, and how best to incorporate such information in risk assessment practices is important to all stakeholders. The task of exploring an improved risk assessment approach requires that various disciplines including physiology, toxicology, and risk assessment come together to consider key issues and methodological approaches. The objectives of this workshop include framing important questions to ask when designing and conducting such a risk assessment, assessing what is known about example endogenous chemicals including normal function and homeostatic control, presenting common modes of action (MOAs) where total dose can be considered, and evaluating current risk assessment practices for such chemicals. The workshop will evaluate opportunities for improvement of risk assessment methodology with consideration of more data and improved technologies. The workshop will also engage the toxicology community in a discussion to help define an improved approach to incorporate this knowledge into the risk assessment process for endogenous exposures.

W 3367b Session Overview and Framing the Issue Through Problem Formulation: Risk Assessment Considerations When Assessing Endogenous and Exogenous Exposures to the Same Chemical

W. Farland. *Colorado State University, Fort Collins, CO.*

Endogenous chemicals with exogenous exposures pose a challenge to risk assessment. Sources of endogenous chemicals and how those sources fluctuate and are managed throughout the life-course of humans must be understood and considered in order to assess the implications of total exposure and attributable risk for specific exposures. Problem formulation has been recognized as an integral step in assuring that risk assessments consider the right information to address the questions of decision-makers. The problem formulation establishes the goals, breadth and focus of the risk assessment. In the case of exposures to exogenous chemicals where endogenous levels of the same chemical already exist, the problem formulation must include a strategy for understanding sources and function of normal physiological levels of the chemical and its metabolites, mechanisms of homeostatic control of relative levels of the endogenous and exogenous exposures at critical targets, endpoints of concern at these levels, dose-response characteristics, and data to assist decision-makers on options for management of endogenous and/or exogenous exposures. These considerations allow the development of a conceptual model with hypotheses for how exogenous exposures may impact risk in the presence of existing endogenous exposures. Discussion of case examples, with well-known modes of action, can assist the process of development of principles for problem formulation for a wider array of situations where endogenous exposures to the same chemical complicate risk assessment for exogenous exposures.

W 3368 What Understanding Loss of Normal Homeostatic Control of Endogenous Toxicants and Their Pathways Tells Us about Risk of Exposure

J. Teeguarden. *Pacific Northwest National Laboratory, Richland, WA.*

Biochemical and physiological homeostatic mechanisms exert control over hormone, nutrient, oxidant and endogenous toxicant levels in cells. The adaption of current risk assessment approaches to treat compounds which contribute to existing endogenous exposures may require a shift from a historical focus on understanding the general pharmacokinetics of exogenously administered compounds to a broader understanding of the strength, nature and variability in homeostatic mechanisms contributing to internal doses. Chief among the hurdles encountered by these efforts is the lack of knowledge on endogenous levels of such chemicals, their means of homeostatic control and their normal function(s). The kind of information that is needed is exemplified by compounds like carbon monoxide (CO), hydrogen sulfide, and manganese, where significant information has been developed on endogenous levels and normal functions. Acetaldehyde stands in contrast to these compounds: Measurements in biological systems are challenging, endogenous sources may not be fully understood, but biomarkers of cellular exposure are accurate and sensitive enough to characterize low endogenous levels. Many diseases, including neurodegeneration, hypertension, heart failure, cancer and inflammation, have been linked to specific abnormalities in metabolism and function of these chemicals. Data needs include a quantitative assessment of variability, intracellular biokinetics and homeostatic control mechanisms in support of biologically-based modeling of endogenous chemical dosimetry when assessing exogenous exposures.

W 3369 Understanding and Assessing Modes of Action from Endogenous and Exogenous Exposure: DNA Adducts, DNA-Protein Crosslinks and Mutagenicity

J. Swenberg. *University of North Carolina at Chapel Hill, Chapel Hill, NC.*

DNA adducts and DNA-Protein Cross-links (DPC) represent key events in mutagenesis and carcinogenesis. For many years, they were thought to be solely the product of exposure to chemical carcinogens. In the past 25 years, however, select DNA adducts and DPC were also identified in cellular DNA from tissues of animals and humans not known to be exposed to exogenous carcinogens. Research on three "known human carcinogens": formaldehyde, vinyl chloride (VC), and ethylene oxide (EO), each of which forms DNA adducts identical to endogenous DNA adducts from these chemicals or other cellular processes, illustrates the importance of understanding the relative contributions of exogenous and endogenous chemical exposures. Advances in technology have allowed the development of quantitative data on endogenous adducts and DPC, mutagenicity, and relationships between endogenous and exogenous adducts. Formaldehyde has the richest data set, with quantitative data on endogenous and exogenous DNA adducts and DPC from the same samples and at extremely low levels. We now know that steady-state concentrations of DNA adducts and DPC are reached at ~28 days and that DPC accumulate with longer or higher exposures. These data can be used to inform the current risk assessment on formaldehyde, including tissue differences and the biological plausibility and accuracy of projected risks. More limited data are available for VC and EO. Identical DNA adducts are formed from oxidative stress. With VC, we have again utilized stable isotope VC exposure to differentiate endogenous from exogenous DNA adducts. To date, stable isotope EO exposures have not been done, but clear evidence of both endogenous and exogenous adducts are formed. This presentation will discuss additional areas of potential research that may lead to improved understanding of exogenous and endogenous exposures to the same chemical and, consequently, improve science-based risk assessment for these situations.

W 3370 The Role of Endogenous Oxidative Stress in Understanding and Assessing the Mode of Action of Exogenous Exposure to Toxic Agents

J. Klaunig. *Indiana University, Bloomington, IN.*

Oxidative stress has been implicated as a cause or contributory in a number of disease etiologies including cancer, cardiovascular injury, neurodegeneration, and immune disease. Increase oxidative damage to cellular macromolecules is also an important feature of the normal aging process. In addition, lifestyle factors including diet and smoking modulate the level of oxidative damage in the cell. Under normal conditions, reactive oxygen species (ROS) are formed as a byproduct of metabolism of oxygen in the cell and have important roles in cell signaling and homeostasis. When in excess, ROS can produce a variety of adverse effects including DNA damage, oxidation of lipids, and oxidation of amino acids in proteins and enzymatic co-factors as well as modification of gene expression. For exogenous chemicals oxidative stress may be produced through four mechanisms; 1) directly from the chemical agent, such as with divalent metals that combine chemically in the cell with H2O2 to produce hydroxyl radicals, 2) indirectly through P450 metabolism or interaction with cellular organelles 3) indirectly via inflammatory cell activation (secondary to necrosis and/or activation of macrophages) or 4) via inhibition of cell ROS defense systems including DNA repair and antioxidants. Therefore in considering the evaluation of potential risk from an oxidative stress mode of action for toxicity, one must take into account the endogenous levels of oxidative stress and damage present in the tissue. Thus implications for risk assessment of exogenous exposures to chemicals (including metals (Cu, Fe), PPAR alpha (DEHP) and CAR activators (dieldrin), and acrylonitrile that induce oxidative damage requires an appreciation of endogenous sources as well as exogenous sources of ROS.

3371 Factoring Endogenous Exposures Into the Risk Assessment Process: Current Approaches and Future Challenges

G. Ginsberg. *Connecticut Department of Public Health, Hartford, CT.*

Assessing the risk associated with exogenous exposures in the presence of endogenous levels of the same chemical presents a variety of challenges. On one hand the damage caused by endogenously formed toxicants may be part of a background aging or pathological process to which the exogenously administered chemical can contribute. The NAS report "Science and Decisions" described the importance of considering chemical additivity to endogenous background processes in evaluating human risk for both carcinogens and non-carcinogens. On the other hand, in certain cases the endogenous levels of the toxicant in question can be high relative to the amount administered exogenously. This can raise questions about the dose response at low dose given how much more endogenous formation may occur in this range. However, endogenous formation may be at the limit of repair and homeostatic mechanisms such that the exogenous exposure is along an effective part of its dose response curve rather than starting from a zero baseline. Examples of these principles will include genotoxic agents such as formaldehyde and ethylene oxide in which endogenous formation may contribute to background rates of cancer to which exogenous chemical may further increase risk. A non-cancer example is the formation of a biomarker of airway inflammation, nitric oxide, which has diagnostic utility in asthma and is increased by irritant gases such as formaldehyde. We are still early in our understanding of endogenous formation of toxicants and their interaction with exogenous exposures with key questions in the areas of how exogenous administration can perturb endogenous pools, and differences in compartmentalization and biokinetics of endogenous vs exogenous chemicals. While these uncertainties are substantial, the NAS guidance on consideration of chemical additivity to background processes dictates that we continue to look for ways of bringing this information into the risk assessment paradigm.

3372 Developmental Immunotoxicology—Are We Adequately Evaluating Safety?

L. P. Myers[1] and G. R. Burleson[2]. [1]*US FDA, Silver Spring, MD and* [2]*BRT, Burleson Research Technologies, Inc, Morrisville, NC.*

The developing immune system has been shown to be more vulnerable to many xenobiotics, both environmental chemicals and pharmaceutical agents, than the adult immune system. Care must be taken to select different windows of susceptibility with a thorough knowledge of the differences in the stage of critical developmental windows between humans and rodents. Sensitivity differences are critical in evaluating therapeutics to be used for pregnant females as well as for drugs prescribed for juveniles. Knowledge of the critical windows of exposure will allow an evaluation of immunotoxicity assessments important for both developmental and juvenile immunotoxicological testing. Pediatric investigation plans (PIP) are now required for both the European and the US FDA regulatory agencies, and the proposed strategy must be defined in the PIP. While there is no set way to perform a thorough evaluation, assessment of immunotoxicology should consider the known mechanisms of action of the agent, observations in adult studies, and the developmental age of the intended patient population relative to immune system development.

3373 Developmental Immunotoxicology–The Role of the Microbiome

R. R. Dietert. *Cornell University, Ithaca, NY.*

Evaluation of risk for the developing immune system from exposure to chemicals, drugs, food additives, and other stressors relies on the use of multiple biomarkers often connected to immune function. But some of the challenges have been to predict developmentally programmed effects on the immune system that may appear later in life and to extrapolate from rodent strains with limited genetic backgrounds to diverse human populations. The recent discovery that the immune system must co-mature with a robust, balanced microbiome for effective childhood and adult immune function raises questions about the scope of useful biomarkers for predicting developmental immunotoxicity (DIT). Since microbial genes constitute up to 99% of those in the human superorganism and the immune system is a sensitive physiological target of microbiome alterations, developmental exposures affecting the microbiome need to be considered within the scope of DIT. The microbial metabolonome is particularly significant in immune developmental programming, signaling, and function, and several immune-relevant microbial metabolites are accessible for quantitation and possible use among predictive biomarkers for DIT. Strategies for modeling the co-maturation and interdependency of the infant microbiome and immune system will be considered. This will include a review of the microbial metabolonome in developmental programming of the immune system, the identification of previously unrecognized vulnerable subpopulations (e.g., premature or Cesarean delivered babies), and specific interactions among early life environmental factors (e.g., maternal health, pediatric diet, toxicants, probiotics, physical activity).

3374 Regulatory Concerns for Developmental Immunotoxicology

R. Houghtling. *US FDA, Silver Spring, MD.* Sponsor: L.P. Myers.

During drug development, the need for nonclinical immunotoxicity testing should be considered for any pharmaceutical (small molecule or biologic) that unexpectedly targets or intentionally targets (e.g., MOA) the immune system. Because of PREA (2003), many Sponsors will need to submit an initial pediatric study plan (iPSP) that describes the nonclinical studies completed and planned to support the safety of the proposed pediatric clinical trial. When pediatric studies are requested, and not waived, pharmaceuticals previously identified as unexpectedly or intentionally targeting the immune system should be tested in young and/or juvenile animals. Because of developmental differences among species, Sponsors should take into consideration the species selected, windows of immune system development, and age of the animals at dosing initiation to ensure that the age range of the pediatric population is adequately represented. The immunotoxicity testing paradigms that need to be included should be discussed with the reviewing Division on a case-by-case basis. Case studies of different strategies employed will be presented and discussed.

3375 Approaches for the Evaluation of Juvenile Toxicology

L. A. Burns-Naas. *Gilead Sciences Inc, Foster City, CA.*

During the development of drugs for pediatric indications, Sponsors often consider the need to conduct studies in juvenile animals. Key questions to be addressed include the relevant species, age and the start of dosing, and the duration of exposure. When the immune system is the primary organ of interest, the species and age questions become increasingly critical to the design and interpretation of studies to assess developmental immunotoxic effects. The general processes for the development of the immune system are generally the same across mammalian species. However, the timing of these events relative to each trimester and birth are different with the rodent being developmentally delayed after birth relative to the human. This presentation will cover these key differences across species and their impact on study design and interpretation. Examples of juvenile studies in multiple species will be provided to illustrate how Sponsors might address the need to assess DIT.

3376 Current Paradigm for Evaluation of Developmental and Juvenile Immunotoxicity Evaluations–Testing and Limitations

G. R. Burleson. *BRT, Burleson Research Technologies, Inc, Morrisville, NC.*

Current testing paradigms for evaluation of developmental and juvenile immunotoxicity are varied and vague. Most evaluations are performed in either mice or rats and obtain data for only one arm of the immune response, i.e., humoral-mediated immunity as measured by the TDAR. The TDAR is the single most important functional means for evaluating immunotoxicity. However, a thorough developmental, juvenile, or adult immunotoxicology evaluation should include all three arms of the immune response: innate, cell-mediated, as well as humoral-mediated immunity. Examples of functional assays to evaluate all three arms of the immune response will be discussed. Functional studies currently available, or under investigation, in non-rodent species will also be discussed and are important for developmental, juvenile, and adult immunotoxicology evaluations to provide data important for a thorough immunotoxicology safety evaluation.

3377a Potential Health and Environmental Effects of Unconventional Hydraulic Fracturing

T. L. Knuckles[1] and J. T. Zelikoff[2]. [1]*West Virginia University, Morgantown, WV and* [2]*New York University Medical Center, Tuxedo, NY.*

Unconventional gas well development in the Marcellus Shale geological formation has reached an all-time high in recent years. As such, many rural communities and regions are experiencing increased industrial activities and possibly air pollutant exposures from shale gas extraction activities. The burgeoning development of unconventional gas well sites has the potential to contribute to poor outdoor and indoor air and water quality. In particular, concentrations of particulate matter (PM) in the fine (<2.5 um, PM2.5) and ultrafine (<0.1 um, PM0.1) size ranges and volatile organic compounds (VOCs) may be increased in areas surrounding drilling operations. Furthermore, water quality issues have been reported in areas of hydraulic fracturing activity. However, potential exposures to complex mixtures of toxicants and the health effects on workers and on nearby residents are poorly understood. This multi-faceted symposium will begin with a discussion on the basics of hydraulic fracturing and the potential occupational exposures. Next will be a presentation on the regulatory policies and proposals that are currently in place at the state and federal levels. The third presentation will explore the potential mechanisms of exposure to the surrounding communities. A talk on air and water chemistry and the exposome will follow, and the workshop will conclude with relevant findings in animal models on the toxicological impacts of air and water samples collected on/nearby a drilling site. This cutting-edge presentation will provide the audience with a chance to better understand unconventional gas and oil drilling and reach their own conclusions on this topic.

3377b Overview of Unconventional Oil and Gas Exploration and Production and Possible Occupational Exposures

J. Snawder. *National Institute for Occupational Safety and Health, Cincinnati, OH.*

Unconventional oil and gas exploration and production (E&P) combines horizontal drilling and hydraulic fracturing processes to liberate oil and gas from tight geological formations. Upstream oil and gas activities include site construction, well drilling, well completions, extraction and well production services. Activities in each segment may present occupational exposure hazards inherent to a given stage of well development. The National Institute for Occupational Safety and Health (NIOSH), with the cooperation of oil and gas industry participation, is conducting research to assess chemical exposure hazards to upstream oil and gas workers. Prior to this work, little was known regarding the variety and magnitude of chemical exposures to these workers. NIOSH research is focused on: 1) identifying processes and activities where chemical exposures could occur; 2) characterizing potential exposures to vapors, gases, particulates and fumes (e.g., solvents, diesel particulate, crystalline silica, acids, metals, aldehydes, and possibly other chemicals identified during the study); and 3) recommending safe work practices and/or proposing and evaluating exposure controls (including engineering controls, substitution, and personal protective equipment). This presentation, an overview of NIOSH research in upstream Oil and Gas production, will highlight current field research efforts in exposure assessment, compare exposures by task, well type and basin, present some preliminary results from NIOSH laboratory research, and outline future research efforts.

3378 Current State and Federal Regulatory Policies and Proposals Regarding Unconventional Natural Gas Drilling, and the Environmental Impacts of Drill Cuttings

T. Polen. *West Virginia Department of Environmental Protection, Charleston, WV.* Sponsor: T. Knuckles.

The proliferation of unconventional natural gas drilling has created concerns related to the environmental impact of these operations. Regulatory agencies (both state and federal) have worked to keep statutes and codes relevant and up to date during this rapidly changing energy landscape. As researchers and regulators work together to assure the environmental, safety, and health of the public; the air, water, drilling, waste and other regulations must be modified. This ever changing regulatory landscape will be explored with specific regulatory details and analysis from the West Virginia, Pennsylvania, and Ohio regulatory agencies, as well as the United States Environmental Protection Agency. Additionally, the results of a yearlong study authorized by the WV Department of Environmental Protection regarding the hazardous characteristics, surface/groundwater impacts of the leachate from drill cuttings disposal, and the feasibility of viable alternatives to their disposal will be presented. This study used exo-toxicity analysis, captured and analyzed samples, existing required data submittals, statistical methods, and economic analysis to investigate this aspect of waste disposal from unconventional natural gas development.

3379 An Approach to Studying Potential Acute and Chronic Disease Associated with Unconventional Natural Gas Development Operations

M. McCawley. *West Virginia University, Morgantown, WV.*

Unconventional natural gas development uses horizontal drilling in conjunction with hydraulic fracturing to gain access to natural gas deposits which may be tightly held in shale deposits and unavailable to conventional vertical drilling operations. The intensive work required to extract this source of energy results in as many as 10,000 vehicle trips per well site, potential release of emissions from those vehicles in congested zones surrounding the drill site and release of other contaminants from materials drawn back out of the borehole after fracturing of the shale. Typical air contaminants would be ultrafine particles from diesels at concentrations exceeding 20,000 p/cc and volatile organic compounds such as benzene ranging from 10 to 270 micrograms per cubic meter. As a result, a rise in upper respiratory disease from 18 to 39% has been seen with closer proximity to the process in nearby communities. Other potential acute effects could include cardiovascular and neurological changes. Contamination of water and subsequent health effects may present a greater challenge for correlation of field data. Because of the relatively short time this process has been used and the extent it is currently being employed, it has not previously been possible to draw detailed conclusions about the chronic hazards that may be posed, although ultrafine particle concentrations of 10,000 p/cc have been associated with disease around mountain top removal activities. Acute conditions, though, may be possible to document and lead to establishment of registries in which to perform surveillance for chronic effects. This combination, of the short duration of drilling and hydraulic fractioning work and the decade's long production of gas from the well, presents unique challenges to regulators and policy makers.

3380 Hydraulic Fracturing, Chemical Constituents, and the Exposome

B. Yan. *Columbia University, Palisades, NY.* Sponsor: T. Knuckles.

Unconventional natural gas, including those developed by hydraulic fracturing, has the potential to serve as a "bridge fuel" to a less carbon-intensive future when compared to coal and liquid fuels. However, there are many challenges, that may include unintended consequences on the environment in communities in which hydraulic fracturing is occurring. The public disputes on hydraulic fracturing have been polarizing and scientific research, especially studies on environmental impacts, has been lagging behind the rapid application of these relatively new techniques. Three major reasons contribute to these sizzling disputes: 1) lack of air and water baseline data before the gas development; 2) limited scientific data with reliable sampling approaches, verified analytical methods, and unbiased data analysis; 3) bias in the selection and use of scientific data. In this talk, we will outline specific air and water pollutants (e.g., brines, heavy metals and PAHs) associated with hydraulic fracturing activities (e.g., methanol) and their possible environmental behaviors and exposure pathways. Additionally, we will detail our recent findings from Pennsylvania, compare our results with others, and suggest future research recommendations.

3381 Wide-Ranging Toxicological Effects of Produced Water from Hydraulic Fracturing in a Mouse Model

J. T. Zelikoff. *New York University Medical Center, Tuxedo, NY.*

Hydraulic fracturing is a novel method of natural gas extraction with unknown risks to human health. One public health consideration of "hydrofracturing" is the wastewater that returns to the surface during the life of the well. Water containing excess proppant that returns during hydraulic fracturing and immediately afterward is called flowback water. Water that moves back through the wellhead with the gas is called produced water. Despite the toxic/immunotoxic effects of the individual produced water constituents (organic compounds, heavy metals, and radionuclides), potential health effects of exposure are poorly un-

derstood. Risks for human exposure could occur via spills, breaches in well casings, or failure of storage pond liners, which ultimately could contaminate local surface and groundwater sources. The goal of our *in vivo* studies was to examine the general toxicological potential, with particular emphasis on immune tumor surveillance mechanisms of produced water in a mouse model. For these studies, a combination of flowback and produced water was used, though it will be referred to as simply produced water. Adult male mice, exposed daily for 3-wk to produced water by oral gavage, were then either injected locally (s.c.) with cultured lymphoma cells and tumor incidence/growth/time to tumor formation measured for 30 days, or tissues collected from unchallenged mice to assess cell profiles, lymphoproliferation and histopathology. Results demonstrated that mice exposed to produced water and subsequently injected with tumor cells had a significant increase in tumor incidence and growth, as well as depleted liver glycogen stores compared to stream water-gavaged control mice. Mice exposed to produced water alone demonstrated a significant decrease in relative liver and iliac lymph node weight and a significant decrease in relative testis weight. These results demonstrate the toxicity of oral exposure of mice to hydraulic fracturing wastewater and clearly demonstrate the need for additional toxicological studies to better identify the health implications for both gas company workers and the public should containment fail.

W 3382 Which Human Cell Lines Should I Use? Choosing the Appropriate Biological Systems for High-Throughput Toxicity Testing

N. S. Sipes[1] and F. Shah[2]. [1]*NTP/NIEHS, Research Triangle Park, NC and* [2]*Pfizer, Cambridge, MA.*

The advent of toxicogenomics, proteomics, and high-throughput screening has provided researchers with massive amounts of information to predict *in vivo* toxicity. Recent examples of such cross-disciplinary initiatives for toxicity prediction include Tox21, ToxCast, QSTAR, and L1000. Retrospectively, to better make sense of *in vitro* to *in vivo* correlation, not only is it important to choose an adequate study design, for example, compound sets and their curated annotations, experimental dose, and time points, but also the choice of suitable cell systems that is relevant to the toxicity phenotypes of interest. Cells have different gene and protein expression patterns, metabolic capabilities, and differential perturbations to compound exposure. Approaches to address the *in vivo* toxicity predictions must address the correct cell type(s) for the problem in question. Ultimately, there is no one universal cell line that is predictive of general or all end-organ toxicities; however, it is important to know which combination is perhaps the most predictive for the given phenotype(s) of interest. The purpose of this workshop is to bring experts from pharmaceutical and biotech industries, academic institutions, and government agencies together to discuss the selection and use of human cell models relevant to specific toxicity predictions. Discussions will encompass a broad range of toxicities (cardiotoxicity, drug-induced liver injury), chemicals (pharmaceuticals, environmental), and cell types (cell lines, primary, stem cells). Speakers will also discuss objectives, approaches, technologies, knowledge gaps, and suggestions for future research. This workshop will be of high interest to a broad audience interested in *in vitro* methods to elucidate and predict toxicological outcomes.

W 3383 Strategies for Choosing Biological Diversity for High-Throughput Toxicity Testing

N. S. Sipes. *NTP/NIEHS, Research Triangle Park, NC.*

The Tox21 and ToxCast efforts have concentrated on testing thousands of chemicals in focused high-throughput screening assays. The desire to increase biological coverage and decrease cost in the next phase of Tox21 has led to interest in high throughput toxicogenomics (HTT). One of the primary considerations in using a HTT approach is the selection of cell systems. Our goal in cell systems selection is to capture the broad diversity of biological responses to chemical treatment. For this reason, the selection process must focus on identifying cell systems that reflect broadly the biological diversity of cell types found in the human body. Finding a suitable database to query these profiles across many biological samples and compounds is a challenge due to the limited studies available. We used data from the National Cancer Institute (NCI) CellMiner and Illumina's NextBio body atlas databases to query the toxicological and genetic diversity from 1076 biological samples including tissues, primary cells, stem cells and cell lines. From the NCI CellMiner database, the 60 cell lines over 9 organ types were correlated similarly across growth inhibition profiles from 597 compounds and baseline gene expression profiles from 22000 transcripts. Dissimilarities among cell types were also seen, with leukemia cells exhibiting the most diversity. Evaluating wider biological space with gene expression profiles, cell lines and tissues clustered tightly within their own groups but farthest apart from each other, while primary cells and stem cells appeared to be of intermediate similarity in biological space. Using an algorithm to pick the top cell lines which represent the most diversity, we found a number of cell lines that can best represent biological space. Practical considerations also need to be considered such as biological vs statistical evaluations when time and funds are limited. This approach has revealed insight into biological diversity, which shows promise toward ensuring adequate coverage of biological space toward chemical-biological high-throughput screening.

W 3384 Selecting Cell Types that Provide Transcriptomic Data Over a Broad Range of Modes of Action

G. Daston. *Procter & Gamble, Cincinnati, OH.*

It is possible to identify modes of toxicity through global analysis of gene expression in responsive tissues or cell types. We are interested in using gene expression to identify potential modes of action for large numbers of chemicals, for which wholesale screening of most or all cell types in the body would be impractical. Is it possible for a relatively small number of cell types to provide broad coverage for modes of repeat-dose toxicity? In an initial study using only breast cancer and liver tumor or liver stem cell-derived lines we were able to identify 21 of 22 modes of action. We have conducted a larger study using chemicals representing a broad range of modes of action, along with cell types that we expect to be responsive to at least some of these. The approximately 150 chemicals are a combination of pharmaceutical agents (e.g., neurotransmitter receptor agonists, ion channel blockers, etc.) , which are expected to have high specificity, and industrial chemicals (e.g., glycol ethers, plasticizers, solvents, etc.) selected from ToxCast, which are expected to have less target specificity. Selection of modes of action is biased towards those that are governed by weak interactions (e.g., receptor binding, reversible interactions with enzymes, signaling proteins, ion channels, etc.). Molecules that act via reactive chemistry have been largely limited to those that require metabolic activation, thereby providing a separate challenge to our cell selection. Chemicals were tested at three concentrations ranging two orders of magnitude. Data were originally generated in two platforms, a global gene array and a smaller array (L1000) for which genomic gene expression can be inferred, with comparable results. Subsequent data was collected in L1000 only. Cell types were selected because they express a large number of receptors of the nuclear hormone superfamily, have some reported xenobiotic metabolizing capability, and/or have some special characteristics in maintaining epithelial integrity or excitatory function. These studies show that combinatorially these cell types adequately predict a variety of modes of action.

W 3385 Defining Appropriate *In Vitro* Model Systems for Assessing Toxicity in Early-Product Development

Y. P. Dragan. *Takeda, Cambridge, MA.*

An integrated product profile is essential in guiding the synthesis and selection of agents during early product development. The selection of test system for safety assessment must reflect the question to be answered, the throughput desired, and the required confidence for decision making. The *in vitro* model systems must adequately reflect what is observed *in vivo* (in preclinical species or the intended clinical population). Cell lines are useful when limited metabolism is required, such as for cytotoxicity screening of parent compound, while appropriate culture conditions (matrix, growth factors, cell types, and scaffolding) are required for more functional assessments such as tissue specific protein synthesis for the liver or action potential activity for cardiac cells). When the target is known and the functional, structural, or signaling pathway is intact, then cell lines can provide useful mechanistic insights or in early screening. The lack of consistent and available human cells has led to the use of cell lines and primary cells with limited functional activity, but adequate responsiveness to examine selected endpoints. More recently human iPSC-derived cardiomyocytes that reflect aspects of *in vivo* human response have become commercially available. Gene expression analysis, the expression of cardiac maturing structural proteins, and the dose dependent response to a set of 50 positive and negative ionotropes provide confidence in the utility of iPSC derived cells for early safety screens. Since the choice between cell lines, primary cells, or stem cells, is dependent on the translatability of the functional response, an adequately characterized set of agents was compared across each model system to determine strengths and weaknesses of each for translation to preclinical and ultimately clinical response. Functional assessment in cardiomyocytes-derived from iPSC cells using label-free methods measures provide an example for assessment of safety across the preclinical through clinical safety continuum and have been retrospectively validated to current models and clinical response.

3386 High-Throughput Toxicity Testing–What Do Primary Human Cell-Based Assays Provide?

E. L. Berg. *BioSeek, a division of DiscoveRx, South San Francisco, CA.*

We are building a large reference database of drug and chemical activity profiles tested in a panel of primary human cell-based assays, BioMAP systems, for analyzing compound profiling projects for academic collaborators, industry partners, and for the United States EPA, as part of the ToxCast program. For this database, concentration-dependent effects of tested agents on the levels of disease and tissue-relevant protein biomarkers are measured from a diverse range of cell types including endothelial, epithelial, smooth muscle and immune cells, dermal and lung fibroblasts, macrophages, keratinocytes, and hepatocytes, among others. Reproducible activity patterns have been identified that correlate with specific targets and pathways as well as cellular mechanisms of toxicity. We have further identified selected assays and endpoints that are associated with organ specific toxicities, including toxicities of the skin, vasculature and liver. Expression of cell surface VCAM-1 in a fibroblast model of wound healing, the BioMAP HDF3CGF system, is associated with skin side effects induced by MEK kinase inhibitors (e.g. trametinib, pimasertib and selumetinib), p38 MAPK inhibitors, and the IL-1 receptor antagonist, anakinra. The potential to induce thrombosis-related side effects (associated with sirolimus, selective estrogen receptor modulators and certain second generation anti-psychotics) correlates with increased levels of tissue factor (CD142) in an endothelial cell-based inflammation system, the BioMAP 3C system, involving the process of vascular cell autophagy. Drugs associated with steatotic hepatitis (amiodarone, tamoxifen and aplaviroc) are selectively active in vascular cell types, endothelial cells and arterial smooth muscle cells, and share activities related to cell health and cholesterol metabolism, activities that show utility as potential sentinel assays for predicting drug induced liver injury. This data-driven approach has improved our understanding of toxicity mechanisms underlying adverse drug reactions and is helping to support the construction of adverse outcome pathway (AOP) frameworks.

3387 Constructing the LINCS Reference Panel of Cell Types to Characterize Small-Molecule Pleiotropy

A. Subramanian. *Broad Institute, Cambridge, MA.* Sponsor: N.S. Sipes.

A key challenge in therapeutic development is optimizing the desired activity of a small-molecule drug while minimizing its off-target effects. Building upon past work in the Connectivity Map project, we hypothesized that perturbations in mammalian cell culture might provide an approach that is truly generalizable, systematic, and provide a biologically relevant readout of the drugs activity – on and off-target. As part of the NIH LINCS program, the Broad Institute has generated 2.1M gene expression profiles from thousands of small molecule compounds in 18 cell types, including cancer and stem cell lines, normal immortalized cells, and primary cells. Our analysis of this unprecedented dataset revealed notable differences in response between cell types in several classes of drugs thus suggesting the need to assess activity over a range of cellular contexts to fully characterize a drugs effect. We will present a methodology that given a drug that has been profiled in the 18 cell types, allows for estimation of off-target effects by comparing the signatures to themselves and to a reference database of ~3,000 shRNA and CRISPR-based signatures. These analyses found newly revealed activity in 22 of the 67 drug classes studied. Secondly, we will address the issue of optimizing the cell line panel for such studies, including the background behind choosing the cell types and what the data tells us using the LINCS resources. In particular, we profiled a collection of 312 drugs in 47 cell types spanning 14 distinct lineages. We then applied a novel greedy algorithm-based approach that optimized a cost function derived from a minimal spanning distance tree to identify the subset of cell types that can fully characterize the diversity of responses to a small-molecule perturbagen.

3388 Development of a Robust 3D Extracellular Matrix to Support an Organotypic Model of Human Mammary Ducts

M. K. Livingston, M. M. Morgan, W. T. Daly, B. P. Johnson, K. E. Sung, L. A. Schuler, W. L. Murphy and D. J. Beebe. *Biomedical Engineering, University of Wisconsin - Madison, Madison, WI; Chemistry, University of Wisconsin - Madison, Madison, WI; Comparative Biosciences, University of Wisconsin - Madison, Madison, WI; Molecular and Environmental Toxicology Center, University of Wisconsin - Madison, Madison, WI.*

We've developed a microfluidic mammary duct model that attempts to balance the goal of matching *in vivo* conditions with the needs of a robust screening model. This model will be used to determine the ability of environmental toxicants to effect different stages of ER(+) ductal breast cancer: normal, atypical ductal hyperplasia, ductal carcinoma *in situ*, and invasive ductal carcinoma. We've attempted to match the extracellular matrix (ECM) materials in the ductal model to those ECMs found *in vivo* at each stage. We have tested both natural and synthetic ECM materials in the 3D model. Natural hydrogels were made from collagen I, collagen IV, fibrinogen, or laminin. Preliminary results suggest that stiffer natural matrices generally improve adherence, confluence, and morphology of mammary epithelial cells in the duct. Synthetic poly(ethylene glycol) (PEG) hydrogels coupled to protein sequences can also be used as ECM to mimic natural fibers with reduced inherent variability. A 2D screen of 152 PEG hydrogel variants identified candidate substrates by adherence of normal and tumorigenic cell lines, MCF12a and MCF7. In 2D, we found that PEG hydrogels that incorporated matrix metalloproteinase cleavable cross linkers scored high in adherence whereas those PEG hydrogels that incorporated a non-cleavable cross linker scored low, regardless of adhesion sequence content. Each PEG hydrogel that scored high in the 2D screen was then used in the 3D model and monitored for cell adhesion, tissue-like function, and cell viability in the following categories: % confluence of cell monolayer, staining of e-cadherin, f-actin, and polarity, and viability staining. Results from the 3D screen of PEG hydrogels suggest that cleavable cross linkers are also important in the 3D duct model. Further optimization of materials will aid development of a toxicant screening platform that models four representative stages of breast cancer with corresponding ECM conditions.

3389 Differences in the Rate of *In Situ* Mammary Gland Development of Three Strains of Rat Used in Toxicity Testing Studies: Implications for Timing of Mammary Carcinogen Exposure

J. P. Stanko[2], G. E. Kissling[1] and S. E. Fenton[2]. [1]*NIEHS, Research Triangle Park, NC and* [2]*Division of the National Toxicology Program, National Toxicology Program Laboratory, Research Triangle Park, NC.*

The potential of chemicals to alter susceptibility to mammary tumors is typically assessed using an induced breast cancer model in various rat strains. Chemical induction of mammary tumors is achieved by administering a specific carcinogen at a lifestage when the glands are most sensitive to tumorigenicity. Therefore, the rate of mammary gland development must be considered so the timing of carcinogen administration is impactful. In this study, *in situ* mammary gland (MG) development was assessed in the Harlan Sprague Dawley (Hsd:SD), the Charles River Sprague Dawley (Crl:SD), and the Charles River Long Evans (Crl:LE) strains of rat at postnatal day (PND) 25, 33, and 45. Though body weight was consistently lower at all time points and day of vaginal opening occurred later in female Hsd:SD rats, our data suggest MG development is accelerated in pre-and peripubertal Hsd:SD rats. A quantitative Sholl analysis revealed significantly more branching intersections, greater epithelial area and fuller epithelial growth in Hsd:SD MG at PND25. Convergence of inguinal MGs had occurred in 33% of Hsd:SD rats at PND25 and in 100% at PND33 while it had not occurred in any of the Crl:SD or Crl:LE rats at PND25 and in <45% of MG in those strains at PND33. Developmental scores of Hsd:SD MG were significantly higher than those of Crl:SD and Crl:LE at PND25 and PND33 and were higher than Crl:LE at PND45. However, at PND33, MG of Crl:SD and Crl:LE rats exhibited significantly more terminal end buds (TEBs) and TEB/mm than MG of Hsd:SD rats. These data suggest that in mammary tumor-induction studies employing these strains, administration of the carcinogen should be timed appropriately, based on strain, to specifically target the time of peak TEB occurrence.

3390 Perinatal Exposure to Brominated Flame Retardants (BFRs) Suppresses E-Cadherin and THRα in Mammary Glands at Puberty

M. Lavoie[2], E. Dianati[2], M. Wade[1], B. Hales[3] and I. Plante[2]. [1]*Health Canada, Ottawa, ON, Canada;* [2]*INRS Institut Armand Frappier, Laval, QC, Canada and* [3]*McGill University, Montreal, QC, Canada.* Sponsor: I. Plante.

Brominated flame retardants (BFRs) are chemicals widely used in consumer products including electronics, vehicles, plastics and textiles to reduce ignition rates. Despite restriction of major BFRs throughout the world, mainly due to their persistant and bioaccumulative nature, human exposure persists due to release from existing consumer items. The contribution of BFR exposures to breast developmental anomalies and cancer, as well as the mechanisms implicated in the toxicity of these chemicals remain poorly understood. This project aims to assess the effects of perinatal exposure to BFRs on mammary gland development and determine mechanisms involved in BFRs toxicity. We focused particularly on the role of intercellular junction proteins, which are critical for mammary gland function and development. Female rats were exposed through diet to an environmentally relevant mixture of BFRs two weeks prior to mating, during gestation and during lactation. Female offspring were sampled at critical stages of mammary gland development including weaning (post-natal day 21), puberty (PND46) and maturity (PND208). BFRs treatment had no significant effect on total body weight or mammary gland weight nor did it affect the epithelial surface area or the elongation of the lactiferous ducts. E-cadherin protein expression was significantly downregulated in the PND46 rats that received the lowest BFR dose. The remaining junctional proteins were not significantly affected by BFR treatment. Thyroid hormone receptor alpha (THRα) was also significantly downregulated at puberty (PND46) in the rats that received the lowest dose of the BFR mixture. The downregulation of THRα may indicate a disturbance in thyroid signaling caused by BFR treatment while the suppression of E-cadherin protein expression suggests that adherence junctions might be affected by a perinatal exposure to these compounds. Financed by NSERC and FRQS (IP), by FUAFI (ML), CIHR (ML, BH, MW) and Health Canada CMP (MW).

3391 Comparison of Quantitative Estrogen Profiles in Plasmas and Mammary Glands of Women with Those in a Rat Model of Estrogen-Induced Tumorigenesis

D. Pemp[2], C. Kleider[2], F. J. Möller[3], N. Iva[1], G. Vollmer[3], H. L. Esch[2] and L. Leane[2]. [1]*University Hospital of Würzburg, Würzburg, Germany;* [2]*Department of Food Chemistry, University of Würzburg, Würzburg, Germany and* [3]*Molecular Cell Physiology and Endocrinology, TU Dresden, Dresden, Germany.*

Although August Copenhagen Irish (ACI) rats with 17β-estradiol (E2)-releasing implants are a accepted model to study the etiology of breast cancer, up to now estrogen profiles and levels generated with specific methods of analysis have not been compared with the human situation. Thus, E2, estrone (E1), their methylated catechols and E1-sulfate (E1-S) were determined in plasma and mammary gland (MG) tissue of a) women (age 18-66) not taking estrogen-active drugs undergoing mammoplasty, b) ACI rats without (ACI-E2) and c) with silastic implant containing 3 mg E2 (ACI+E2) by GC- and UHPLC-MS/MS using deuterated estrogens. Human (h)MG tissues were categorized histologically into those prior to (preReg) and after (postReg) age-related regression preceding menopause. All ACI+E2 developed tumors in at least one MG and both tumors and macroscopic non-tumor tissues were analyzed. 2-Methoxy-E1 was the only methylated catechol detected, occurring in <10% of human and ACI-E2 samples as well as ACI+E2 plasmas. Yet, in up to 90% of MG in ACI+E2 25-375 fmol/g were detected. E2 plasma levels were highest in preReg women (n=7)=ACI+E2 (n=19)>postReg (n=6)=ACI-E2 (n=38). In contrast, E2 tissue levels in hMG (n=36) and ACI-E2 (n=30) were comparable but were 10 times higher in ACI+E2 (n=32). MG/plasma ratios of E2 were lower in humans than in ACI rats, whereas ACI groups did not differ. Ranking of E1 levels in plasmas and MG was human>ACI+E2>ACI-E2 and ACI+E2(non-tumor)>tumor=hMG>ACI-E2, respectively. MG/plasma ratios of E1 were highest in ACI-E2 and ACI+E2(non-tumor)>tumor>human. E2/E1 ratios in plasma and MG were significantly lower in humans than in ACI-E2. Exposure of rats to E2 did not affect E2/E1 in plasma but increased the median ratio in MG. In addition, E2/E1 was higher in tumors than in non-tumor MG of ACI+E2. Most strikingly, whereas E1-S was quantifiable in all human samples, no E1-S was detected in ACI rats. Taken together, most levels, ratios and even qualitative occurrences differ significantly between ACI and humans. Supported by DFG LE-1329/10-1.

3392 Circadian Regulation of PAH Metabolism in Breast Cancer and Mouse Mammary Gland

E. E. Schmitt, R. Barhoumi Mouneimne, R. Metz and W. Porter. *Veterinary Integrative Biosciences, Texas A&M University, College Station, TX.* Sponsor: S. Safe.

The circadian clock plays a key role in coordinating many biological processes from behaviors to cellular metabolism and mitosis. Despite the fact that circadian rhythms are associated with critical biological processes, very little is known about its mechanistic role in carcinogen metabolism, endocrine disruptor activity and mammary carcinogenesis. Furthermore, recent studies have linked disruption of circadian rhythms with increased breast cancer risk, suggesting women working alternative shifts may have a higher risk of breast cancer. We have found that time of day, as well as individual circadian clock components, uniquely regulate processes governing benzo-a-pyrene (BaP) activated aryl hydrocarbon receptor (AHR) and estrogen receptor (ER) mediated endocrine disruptor (EDC) signaling in the mouse mammary gland and breast cancer cell lines. Knockdown of PER1 or PER2, key transcriptional regulators of the molecular clock, in MCF10A normal breast and MCF7 breast cancer cells, resulted in an increase and phase shift in CYP1A1 and ER-mediated gene expression. Furthermore, we analyzed simultaneous changes in at least 8 BaP metabolites using a novel multiphoton live cell imaging approach with advanced linear spectral unmixing and showed an elevation in BAP adducts and increased DNA damage. In the mouse mammary gland we found that BAP induction of Cyp1a1 and Cyp1b1 is influenced by time of day in WT animals, is hyper-induced in Per mutant mice and differentially activates ER-dependent mammary epithelial cell proliferation. Therefore, time of exposure or disruption of circadian rhythms through shift work or individual clock components increases susceptibility to BaP mammary carcinogen activity through the interaction of multiple pathways including metabolism, signaling and DNA damage response and repair.

3393 Neonicotinoids Induce a Promoter-Switch in CYP19 Expression in Breast-Cancer Cells and Alter Aromatase Activity and Hormone Production in a Feto-placental Co-Culture Model

E. Caron-Beaudoin[1], M. Denison[2] and T. Sanderson[1]. [1]*INRS-Institut Armand-Frappier, Laval, QC, Canada and* [2]*Department of Environmental Toxicology, University of California in Davis, Davis, CA.*

In estrogen-dependent breast cancer, CYP19 expression is increased via activation of several promoters (PII, I.3, I.7) and by inhibition of the normal I.4 promoter. CYP19 (aromatase) biosynthesizes estrogens, which are also involved in development of the placenta, where CYP19 is expressed via its I.1 promoter. Little is known about the endocrine disrupting potential of neonicotinoid insecticides on CYP19 expression. Objectives were to develop human cell-based assays to identify chemicals that alter promoter-specific expression of CYP19, and to determine the effects of neonicotinoids in these cell assays, which included a breast cancer model and a co-culture model of the feto-placental steroidogenic unit. H295R adrenocortical carcinoma, Hs578t breast cancer cells, BeWo trophoblast cells and a co-culture of H295R and BeWo cells were exposed to atrazine or neonicotinoids (imidacloprid, thiacloprid, thiamethoxam) for 24h. CYP19 expression was measured by RT-qPCR and aromatase activity by tritiated water-release method. Estradiol, estrone, DHEA and B-hCG production in co-culture was determined by ELISA. In H295R cells, atrazine concentration-dependently increased PII- and I.3-mediated CYP19 gene expression (7-fold) and aromatase activity (2-fold). Thiacloprid and thiamethoxam (0.1, 0.3, 10μM) significantly induced PII- and I.3-mediated CYP19 expression (14-fold) and aromatase activity. In Hs578t cells, thiacloprid and imidacloprid inhibited I.4- and increased PII-mediated CYP19 expression (up to 50-fold), resulting in elevated aromatase activity. In BeWo cells, all three neonicotinoids inhibited I.1-mediated CYP19 expression. In the co-culture, the neonicotinoids increased estrogen production. Atrazine and neonicotinoids alter CYP19 gene expression, aromatase activity and estrogen biosynthesis in a promoter-specific manner. We are the first to provide a mechanistic basis for the potential of neonicotinoids to alter the biosynthesis of estrogens tissue-specifically via alternate CYP19 promoters active in placenta or in hormone-dependent breast cancer.

3395 Qualification of Inhalation Exposure Systems for Conventional and Electronic Cigarettes

H. Irshad, P. Kuehl, D. Kracko, B. C. Moeller, E. Yates and J. McDonald. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

A large body of literature is available on conventional cigarettes smoke toxicity and inhalation exposures systems. However, there is a paucity of data on the qualification of inhalation exposure systems for *in vivo* exposure to conventional cigarette smoke. With the recent increasing popularity of electronic cigarettes, there is a new found interest in the characterization of tobacco products including electronic cigarettes and comparison of inhalation toxicity of conventional and electronic cigarettes. Characterization of a whole-body cigarette smoke inhalation exposure system was performed by conducting qualification tests and characterizing the physical and chemical properties of the cigarette smoke. Qualification of the inhalation exposure system was conducted according to a qualification plan that defined the performance criteria. The exposure system qualification tests were performed at 100-mg/m3 and 250-mg/m3 cigarette smoke mass concentrations. The exposure system was tested for the uniformity of aerosol mass concentration (target co-efficient of variation <25%) and stability of aerosol concentration over two hour (target co-efficient of variation < 25%). These tests were performed using a 1-m3 internal volume (H-1000) whole body exposure chamber. Conventional cigarette smoke was generated using an AMESA cigarette smoke machine using Type 3R4F (filtered) research cigarettes. The exposure environment was characterized for mass concentration and the following compounds: nicotine (4.93 mg/m3), cotinine (52.9 µg/m3), ammonia (0.42 ppm), formaldehyde, benzene (35.6 ppbv), toluene (64 ppbv) and carbon monoxide. In addition particle size distribution of the aerosol was measured using TSI Aerodynamic Particle Sizer (NMAD 0.67 micromemetr) and TSI Fast Mobility Particle Size (NMAD 93 nanometer). The same system was also used to generate electronic cigarette aerosol using 'blu' e-cigarettes. The aerosol from electronic cigarette was also characterized for the parameters mentioned above. This poster will describe the materials and methods and results of these tests.

3396 Molecular Impact of Electronic Cigarette Exposure on Human Bronchial Epithelium *In Vitro*

E. Moses[1], T. Wang[1], G. R. Jackson[3], E. Drizik[1], C. Perdomo[1], D. Brooks[2], G. O'Connor[1], S. Dubinett[4], P. J. Hayden[3], M. Lenburg[1] and A. Spira[1]. *[1]Boston University School of Medicine, Boston, MA; [2]Boston University School of Public Health, Boston, MA; [3]MatTek Corporation, Ashland, MA and [4]UCLA, Los Angeles, CA.*

Background: Electronic cigarettes (ECIGs) are an emerging alternative tobacco product thought to be safer than traditional tobacco cigarettes (TCIGs). Despite the increasing prevalence of ECIG use, few studies have evaluated the potential physiological effects of ECIG exposure. Objectives: In this study we aimed to determine the global gene expression effects of ECIG exposure on bronchial epithelium *in vitro*. Methods: Human bronchial epithelial cells (HBECs) grown at Air Liquid Interface (ALI) (EpiAirway) were exposed to direct ECIG vapor or TCIG smoke. ECIG vapor exposures included tobacco and menthol flavor with and without nicotine. Results: We found that while some gene expression changes induced by ECIG exposure are shared with TCIGs, ECIGs have a distinct effect on airway epithelial cells, inducing gene pathways related to cell cycle and mitosis. Of particular interest, ECIG exposure induces the expression of genes involved in oxidative and xenobiotic stress pathways, similar to, but generally lower in magnitude, than the effects of TCIGs. Furthermore, ECIG exposure decreases the expression of genes involved in cilia assembly and movement. Conclusions: These results indicate that ECIG vapor may induce significant cellular stress and molecular alterations within airway epithelium. These results underscore the urgent need for further studies to fully characterize the type and magnitude of cellular and molecular responses to ECIG exposure, in order to determine whether their use may lead to deleterious health outcomes.

3397 Acute Effects of Electronic Nicotine Delivery Systems (ENDS) on Bronchial Epithelial Cells

N. J. Leigh[2], R. I. Lawton[2], E. M. McConnaghy[2], P. A. Hershberger[2] and M. L. Goniewicz[2]. *[1]Department of Health Behavior, Roswell Park Cancer Institute, Buffalo, NY and [2]Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, NY.*

Significance: Electronic nicotine delivery systems (ENDS) are designed to deliver nicotine-containing aerosol via inhalation. These products vary greatly in size, battery power, nicotine concentration, solvent composition, and flavoring. We examined how ENDS characteristics affect potential acute inhalation toxicity of these products. Methods: Using an air-liquid interface system, we exposed H292 bronchial epithelial cells to 55 puffs of ENDS aerosol generated with a smoking machine. We generated aerosol from: A) various models and types of ENDS and B) one ENDS product with variable 1) nicotine concentrations; 2) nicotine solvents; 3) device power and 4) flavorings. We quantified *in vitro* toxicological effects of ENDS using trypan blue assay (for cytotoxicity), neutral red uptake assay (for cell viability) and an interleukin-6 ELISA (IL-6, to quantify inflammatory reaction). Results: We found that product characteristics significantly affect cytotoxicity of an ENDS aerosol. The cell mortality (%) varied from 20.0±8.6 (SD) to 67.8±7.0, and inhibition of cell viability (%) varied from 4.7±2.8 to 97.5±2.6 across tested ENDS types ($p<0.05$). Increasing device power resulted in increased toxicity of the aerosol: cell mortality decreased from 88.2±3.2 at 3.3V to 36.6±2.75 at 4.8V and inhibition of cell viability changed from 21.9±3.1 to 63.1±1.8, respectively ($p<0.05$). Aerosol generated from strawberry-flavored product was most cytotoxic, and aerosol from tobacco-flavored product was least cytotoxic (cell mortality 82.8±2.2 vs. 36.6±2.0 and inhibition of cell viability by 53.8±2.9 vs. 21.9±2.2; $p<0.05$, respectively). Exposure to aerosols did not result in significant IL-6 release. Conclusions: Flavorings used in ENDS and the device power significantly affect acute cytotoxicity of generated aerosol. Future studies are needed to investigate long term effects of exposure to ENDS products among users of these products, including those who quit or continue to smoke conventional cigarettes (dual users), as well as those who never smoked tobacco cigarettes.

3398 Mitochondrial Oxidative Stress Is Associated with Mitophagy in Human Lung Cells Following Direct Exposure to Electronic Cigarette Aerosols

C. A. Lerner, P. Rutagarama, I. Sundar and I. Rahman. *Environmental Medicine, University of Rochester, Rochester, NY.*

Lack of scientific knowledge regarding the potential deleterious health effects of Electronic cigarettes (E-cig) devices has resulted in manufactures implying they are safer alternatives to conventional smoking. However, the immediate toxic effects of E-cig aerosol exposure directly to lung cells remain unclear. Mitochondria, being particularly sensitive to environmental oxidants/carbonyls and excessive heat are variably exposed to these factors which are detectable in E-cig aerosols. Previously, we have shown E-cigs are capable of generating reactive oxidants and depleting glutathione in mouse lung, a critical antioxidant. As a potential source of endogenous reactive oxygen species (ROS), we are interested in understanding the role of mitochondrial dysfunction in response to E-cig aerosols. Excessive ROS produced by dysfunctional mitochondria, commonly occurs in conjunction with an inflammatory response. To assess parameters of mitochondrial toxicity in lung cells exposed to E-cig aerosols, we employed an air liquid interface (ALI) system utilizing a specialized trans-well chamber to mimic pulmonary air-liquid flow dynamics during E-cig aerosol exposures. Human lung epithelial cells and fibroblasts (HFL-1) were exposed to E-cig aerosols for 10 min (duration of 4 sec. puff every 30 sec). ALI exposures of HFL-1 exhibit an increase in mitochondrial superoxide (O2-), susceptibility to altered oxidative-phosphylation (OxPhos) protein levels across three different brands of E-cig, and a 2.1 fold increase in the level of total cellular DNA damage measured by the Comet assay. Significant increases in IL-6 and IL-8 release in conditioned media from E-cig aerosol exposed HFL-1 cells confirm that elevated mitochondrial O2- promotes an inflammatory cell state. These results indicate that E-cig aerosols potentiate mitochondrial oxidative stress which is accompanied by engagement of mitophagy (altered mitophagy markers). Our data highlights the potential deleterious oxidative and pro-inflammatory effects of direct E-cig aerosol exposure associated with dysfunctional mitochondria in lung cells. Supported by NIH pulmonary training grant T32 HL066988 and 2R01 HL085613

3399 Electronic and Traditional Tobacco Cigarettes Induce Vascular Endothelial Dysfunction Through Similar Mechanisms

M. A. El-Mahdy[4], O. A. Badary[1,3], Y. A. Alzarie[3,4], C. Hemann[4], M. G. Helal[4], A. Elmahdy[4], R. S. Ismail[2], T. M. Abdelghany[2], A. M. Mansour[2] and J. L. Zweier[4]. *[1]Ain Shams University, Cairo, Egypt; [2]Al-Azhar University, Cairo, Egypt; [3]National Organization of Drug Control and Research, Cairo, Egypt and [4]The Ohio State University, Columbus, OH.* Sponsor: F. Lowe.

Nicotine containing electronic cigarette (e-cig) use has achieved growing popularity and surpassed traditional tobacco cigarettes (TC). The claim that e-cig devices are healthier than TC and help smokers quit is unsubstantiated and warrants further investigation. Research has shown that e-cig vapor (EV) contains banned toxic chemicals that cause cytotoxicity, oxidative stress, and inflammatory response and induce a gene expression signature similar to tobacco smoke. The aim of this work was to investigate whether or not e-cigs cause vascular endothelial dysfunction (VED) similar to that induced by TC, and to characterize the cellular and molecular mechanisms of such effects. Mice were exposed for 32 weeks to EV, TC smoke (4 hrs/day, 5 days/week), or 28 days of infused nicotine (2 mg/kg). The SCIREQ "InExpose" smoking system was used to generate TC smoke (35-ml puff of 2-seconds duration once every 30 seconds) and EV (55-ml puff of 4-seconds duration once every minute) from 3R4F reference research cigarettes and locally available e-cig cartridges containing 2.4% nicotine, respectively. Significant increase of blood pressure was observed following all types of exposure. Impaired acetylcholine (Ach)-induced endothelium-dependent relaxation with a shift to the right and downward in the Ach-concentration response curve was observed in thoracic aortic segments of all exposed groups. Aorta of all exposed groups showed increased reactive oxygen species (ROS) generation, tissue oxidative damage and elevation of protein carbonylation. Tetrahydrobiopterin (BH4) and endothelial nitric oxide synthase (eNOS) expression and phosphorylation were decreased in the aorta homogenate of all exposed groups. Thus, e-cigs, TC, and nicotine induced VED and hypertension, with TC showing the highest effects, through generation of ROS which depleted BH4 and impaired eNOS activation. Overall, our study shows that e-cigs cause toxic effects on vascular endothelial function similar to that induced by TC, and can trigger vascular disease through similar mechanisms.

3400 Toxicological Characterization of the Mentholated Heat-Not-Burn Product THS2.2M in a 90-Day OECD Inhalation Study

A. Oviedo[2], S. Lebrun[1], G. Rodrigo[1], G. Vuillaume[1], J. Ho[2], P. Vanscheeuwijck[1], W. Hayes[3], J. Hoeng[1] and M. Peitsch[1]. *[1]Philip Morris International R&D, Neuchâtel, Switzerland; [2]Philip Morris International Research Laboratories, Singapore, Singapore and [3]Spherix Consulting, Rockville, MD.* Sponsor: W. Hayes.

The toxicity of aerosol from the mentholated Tobacco Heating System (THS2.2M), a candidate modified risk tobacco product (cMRTP), was characterized in Sprague-Dawley rats in a subchronic inhalation study following the OECD Test Guideline 413, and compared to the one inherent to cigarette smoke (CS) from mentholated reference cigarettes (MRC). Rats were nose-only exposed for 6 hours per day, 5 days per week for 90 days to either filtered air, CS from MRC to a target nicotine concentration of 23 µg/L (with 2 menthol concentrations in CS) or to aerosol from THS2.2M to 3 different target nicotine concentrations (15, 23 and 50 µg/L). The analysis of the test atmosphere showed a reduction of up to 90% and 98% in aldehydes and CO, respectively when compared to CS from MRC. Aerosols were reproducibly taken up by the rats as indicated by determination of carboxyhemoglobin and selected aerosol constituents and their metabolites in blood. Exposure to CS resulted in typical smoke related toxicological effects consistent with findings reported in previous studies. Histopathological findings in the respiratory tract and pulmonary inflammation in THS2.2M exposed groups were significantly lower in severity or absent when compared to MRC-exposed groups. This was consistent with reduced inflammatory cells infiltration and cytokine expression in bronchioalveolar lung fluid suggesting reduced inflammatory processes as compared to the response to CS exposure. Overall, the data demonstrate that aerosol generated by THS2.2M causes much lower systemic and respiratory tract toxicity than CS from mentholated combustible cigarettes. Furthermore, no menthol related effects were detected after THS2.2M aerosol or MRC CS-exposure.

3401 Systems Toxicological Evaluation of a Heat-Not-Burn Tobacco Product in a 90-Day OECD Inhalation Study in Comparison to Cigarette Smoke

U. Kogel[1], Y. Xiang[1], E. T. Wong[2], S. Boue[1], F. Martin[1], E. Guedj[1], A. W. Hayes[3], M. C. Peitsch[1], P. Vanscheeuwijck[1] and J. Hoeng[1]. *[1]Philip Morris International R&D, Neuchatel, Switzerland; [2]Philip Morris International Research Laboratories, Pte. Ltd., Singapore and [3]Scientific Advisory Board PMIR&D and, Spherix Consulting, Rockville, MD.*

A 90-day rat inhalation study was performed in accordance with OECD test guideline 413 to characterize potential adverse effects caused by subchronic exposure to the Tobacco Heating System (THS) 2.2 aerosol, a heat-not-burn tobacco product, and to compare with those induced by the smoke generated from the reference cigarette 3R4F. Sprague Dawley rats were exposed for a period of 13 weeks to filtered air, three concentrations of mainstream 3R4F smoke (8, 15, 23 µg/l nicotine), or THS2.2 aerosol (15, 23, 50 µg/l nicotine). The evaluation of all OECD recommended endpoints indicated that aerosol from the THS2.2 caused a significantly reduced systemic and respiratory tract toxicity in comparison with cigarette smoke. The response of OECD endpoints may be only detectable after substantial damage. Therefore, the OECD endpoints were augmented with omics analysis generated from respiratory nasal epithelial (RNE) and lung tissue. Transcriptome analysis resulted in more than 3900 differential expressed genes (DEGs) in the RNE of male rats after 3R4F exposure, but in none after THS2.2 exposure. Gene set enrichment analysis using the KEGG database showed very few significant enrichment in THS2.2 groups. One gene set cluster was found that showed similar high enrichment as in the 3R4F groups. Processes belonged to amino acids, lipids and xenobiotics metabolism. Approximately 800 and 1300 DEGs were detected in lung tissue of male and female rats exposed to 3R4F, respectively. 17 DEGs were found in the lung tissue from THS2.2 exposed female rats. Ingenuity pathway analysis linked those genes to cellular movement and inflammatory responses. Overall, transcriptomics analysis showed a reduced response of THS2.2 on respiratory tissues compared with mainstream cigarette smoke.

3402 Systems Toxicology Analysis of Cardiovascular and Respiratory Endpoints from Apoe-/- Mice Showed Similar Effects When Switching to a Candidate Modified Risk Tobacco Product, THS2.2 or Ceasing Smoking

B. Phillips[1], E. Guedj[2], A. Elamin[2], S. Boue[2], G. Vuillaume[2], F. Martin[2], P. Leroy[2], W. Hayes[3], E. Veljkovic[1], M. Peitsch[2] and J. Hoeng[2]. *[1]Philip Morris International Research Laboratories Pte. Ltd., Singapore, Singapore; [2]Philip Morris International, Philip Morris Products S.A., Neuchatel, Switzerland and [3]Spherix Consulting, Rockville, MD.* Sponsor: W. Hayes.

Cigarette smoking is a risk factor for chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD). ApoE-deficient mice are prone to developing premature atherosclerosis and emphysema making it an ideal model in which both pathologies can be assessed simultaneously. We evaluated the effects of cigarette smoke (CS) from a conventional cigarette (3R4F) and aerosol from Tobacco Heating System 2.2 (THS2.2), a candidate modified risk tobacco product (cMRTP). ApoE-/- mice were exposed for up to 8 months to the test aerosol for 3 hours/day, 5 days/week to a target nicotine concentration of 30 µg/l. After 2 months of exposure to CS, cessation and switching groups were further exposed for up to 6 months to fresh air, or THS2.2, respectively. Multiple markers of disease progression were investigated including atherosclerotic plaque formation, pulmonary inflammation, pulmonary function and lung emphysema. Exposure to CS induced time-dependent molecular, physiological and inflammatory pulmonary responses in ApoE-/- mice consistent with emphysematous changes. The area and volume of atherosclerotic plaques measured in the aortic arches were higher in CS-exposed animals compared to both sham and cMRTP-exposed animals at all time-points. Significant changes in the lung transcriptome and proteome of ApoE-/- mice were observed in response to CS-exposure compared to sham-exposed mice. Smoking cessation and switching to THS2.2 resulted in lower activation levels compared to continuous exposure to CS. Both smoking cessation and switching to the cMRTP halted the rate of disease development as assessed by histopathological and molecular endpoints.

3403 E-Cigarette and Cannabis: Assessment of Passive Exposure

A. Berthet[1], M. De Cesare[2], V. Varlet[3], B. Favrat[4], C. Giroud[3], M. Augsburger[3], N. Concha-Lozano[1] and A. Thomas[3]. *[1]Institute for Work and Health, Epalinges, Switzerland; [2]Unita di Medicina e Psicologia del Traffic, Lugano, Switzerland; [3]Forensic Toxicology and Chemistry Unit, University Centre of Legal Medicine, Lausanne, Switzerland and [4]Psychology and Traffic Medicine Unit, University Centre of Legal Med, Lausanne, Switzerland.*

Recreational use of e-cigarette (e-cig) for consuming cannabis is increasing. One recent method is "cannabis vaping". It consists of inhaling concentrated cannabis in butane hash oil (BHO) diluted in e-liquid or propylene glycol (PG). An e-cig with clearomizer temperature (≈ 150°C) is sufficient to decarboxylate the tetrahydrocannabinol (THC) precursor; tetrahydrocannabinolic acid (THC-A). Passive exposure to cannabis vapors from e-cig might thus be possible. Biomonitoring can be used to assess toxicological risk and medical-legal interpretation bias. Passive THC exposure was assessed in cannabis abstinent and non-smoker volunteers (n=9) using an unventilated exposure chamber (15.6-m3). Passive smoke was generated with a three-channel linear piston-like smoking machine, and three e-cigs were activated consecutively with a 20-second delay between two puffs (puff length of 3 seconds). E-cigs were filled with nicotine-free e-liquids (DEA Calliope, Dea Flavour S.R.L) for the placebo exposure, and with 10% BHO in PG for the verum condition. Volunteers were exposed for 1 h. Blood, hair, saliva, sebum, and urine samples were collected before and immediately at the end of exposure. Urine was sampled 6h and 24h after exposure, blood 24h post-exposure, and hair after 1 month. Cannabinoids, THC and its two main metabolites: 11-OH-THC and THC-COOH were quantified in the matrices for both exposures. Our results showed, no or very low cannabinoids, THC, THC-COOH and 11-OH-THC concentrations in all studied matrices. Passive exposures to cannabis in 10% BHO dilution in e-cig may be present; however, concentrations are negligible compared to joint use.

3404 Polybrominated Diphenyl Ethers (PBDE) and Their Hydroxlyated and Methoxylated Derivatives in Blood from E-waste Recyclers, Commercial Fisherman and Office Workers in the Puget Sound, Washington Region

I. Schultz, S. Cade and L.-J. Kuo. *Pacific NW National Lab, Sequim, WA.*

Synthetic polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants in many consumer products including electronic devices. Important routes of human exposure are contaminated food and contact with dust found in households and workplaces. Structurally related derivatives of PBDEs are the hydroxylated (OH-PBDEs) and methoxylated forms (MeO-PBDEs). Humans can metabolize some PBDEs into the OH-PBDE derivative, which is a concern due to greater health risks associated with OH-PBDEs. However, certain OH-PBDEs and MeO-PBDEs are also marine natural products and it is unclear although likely, that marine fish and shellfish, which bioaccumulate these compounds serve as a vector for human exposures. In this study, we compared approximately 30 different PBDE, OH-PBDEs and MeO-PBDEs in household / workplace dust and blood plasma samples provided by 114 volunteers living in the Puget Sound region of Washington State and working in the commercial fishing, electronic recycling (E-waste) or non-specific office occupations. Prior to blood sampling, a two-week food consumption diary is obtained from each volunteer. Results indicate the sum PBDE levels varied between < 30 and up to 3000 ng/ml ww. The OH-PBDEs were detected in all volunteers varying between < 5 - 800 ng/ml ww. The MeO-PBDEs were detected in most, but not all volunteers varying between 0 - 1000 ng/ml ww. For the large majority of volunteers, the sum PBDE levels exceeded the combined OH-PBDE and MeO-PBDEs. Exceptions to this observation were individuals that consumed the highest amounts of seafood (more than 5 and up to 18 servings / week). Electronic waste recyclers generally consumed low amounts of seafood and had PBDE, OH-PBDE and MeO-PBDE blood levels that were intermediate between seafood consumers and non-E-waste office workers. Dust samples from E-waste sites were particularly enriched with PBDE-209 and PBDE-153 relative to non-E-waste businesses and homes. Initial plasma analysis for PBDE-209 suggest levels are near background and not different among occupations. Supported by NIOSH Grant 1R21OH010259-01A1.

3405 Monte Carlo Analysis of Children's Risks from Chemicals in House Dust: Focus on Phthalates and Flame Retardants

G. L. Ginsberg[1], B. Toal[1] and G. Belleggia[2]. *[1]Connecticut Dept of Public Health, Hartford, CT and [2]University of Connecticut Health Center, Farmington, CT.*

Many chemicals have been detected in house dust reflecting their use in consumer products and the built environment. Young children can receive disproportionate exposure to chemicals in house dust due to their greater time spent on the floor, greater hand to mouth activity and small body size. An emerging aspect of children's health protection is the assessment of exposures and risks stemming from their contact with house dust. The current analysis has developed a house dust database for recent USA studies containing data for phthalates, flame retardants, PCBs, pesticides, and perfluorinated compounds. This exposure information is being combined with toxicology data to prioritize those house dust chemicals which pose the greatest risk and present the greatest uncertainties for children's health. The current presentation provides the analysis for 5 phthalates and 9 halogenated flame retardants. RfDs and cancer potency values were identified with updated searches focusing on toxicology and epidemiology studies that could be the basis for alternative RfDs. Rather than rely upon a single determination, a Monte Carlo approach to non-cancer assessment was used. Updated animal-based and epidemiology-based RfDs were used along with pre-existing RfDs to explore the uncertainty in the toxicology value. A Monte Carlo approach was also used to capture the variability in the house dust data while other exposure parameters (child's body weight and dust ingestion rate) were kept constant. The risk distribution that resulted was used to determine the percentile of the distribution which exceeded a hazard quotient of unity. This was used to rank house dust chemicals. A separate ranking was conducted for carcinogenic chemicals in house dust based upon the percentile of the risk distribution greater than 1 per million. Diethylhexylphthalate (DEHP) received the highest non-cancer rank with 34% of its HQ distribution exceeding unity and a maximum HQ of 38. This was based upon incorporation of the US EPA RfD for DEHP along with two alternative RfDs that are both 7 fold below US EPA's RfD. This assessment of children's risk distributions is being used to prioritize house dust components for further sampling, source identification, and risk characterization. Disclaimer: The views expressed in this abstract are those of the authors and do not represent the policy of the State of Connecticut.

3406 Estimation of Human Percutaneous Uptake for Two Novel Brominated Flame Retardants, 2-Ethylhexyl Tetrabromobenzoate (TBB) and Bis(2-Ethylhexyl) Tetrabromophthalate (TBPH) Using the Parallelogram Method

G. A. Knudsen[2], M. F. Hughes[1], J. M. Sanders[2] and L. S. Birnbaum[2]. *[1]Integrated Systems Toxicology Division, NHEERL, ORD, US EPA, Research Triangle Park, NC and [2]Laboratory of Toxicology & Toxicokinetics, National Cancer Institute, Research Triangle Park, NC.*

2-ethylhexyltetrabromobenzoate (TBB) and bis(2-ethylhexyl)tetrabromophthalate (TBPH) are novel brominated flame retardants (FRs). TBPH is used alone as a plasticizer or in mixtures alongside TBB in polyurethane foam FRs; both are contaminants in the indoor and outdoor environment. In the present study, a parallelogram approach was used predict internal doses for dermally exposed humans. Human or rat skin received 100 nmol of [14C]-TBB or [14C]-TBPH/cm2 in toluene. Treated skin was washed and tape-stripped prior to quantifying [14C]-radioactivity in dosed skin and media (*in vitro*) or excreta, tissues, and skin (*in vivo*). Human skin retained 12% of TBB while 0.2% reached the media. Rat skin was more absorptive (36%) and permeable (2%) *in vitro*, while *in vivo* dosed skin retained 10% and 13% reached systemic circulation by 24 h. TBPH was poorly absorbed, with <0.01% of *in vitro* doses recovered in perfusate. *In vivo*, dosed skin contained 8% of applied TBPH and 1.2% was in excreta or tissues. TBB was completely metabolized to tetrabromobenzoic acid by both human and rat skin; [14C]-radioactivity in TBPH-study perfusates was below the limit of detection. It is clear that TBB can be absorbed and metabolized to tetrabromobenzoic acid by the skin and dermal contact with TBB may represent an important route of systemic exposure. TBPH is less likely to be absorbed dermally. Based on these data, ~7% of TBB and ~2% of TBPH may be dermally bioavailable to humans. This work was supported by the intramural program of NCI/NIH. (This abstract does not represent US EPA policy.)

3407 Human Relevance Assessment of Tetrabromobisphenol-A (TBBPA) Induced Uterine Adenocarcinomas: Mode of Action Dependent on High Dose Molecular Initiating Event (MIE)

D. Wikoff[1], S. Borghoff[2], J. E. Rager[1] and L. C. Haws[1]. *[1]ToxStrategies, Austin, TX and [2]ToxStrategies, Cary, NC.*

TBBPA is a nongenotoxic flame retardant used to improve fire safety in a wide variety of consumer products. Estimated human exposures to TBBPA are very low (<0.00008 mg/kg-day), relative to the doses (500 and 1000 mg/kg-day of TBBPA) administered in a recent bioassay that resulted in uterine tumors in Wistar Han rats following chronic exposure. As part of an effort to characterize the relevance of the uterine tumors to humans, data and biological knowledge relevant to the progression of events associated with TBBPA-induced uterine tumors were organized in an adverse outcome pathway (AOP) framework. Based on a review of possible MOAs for chemically induced uterine tumors and available TBBPA data sets, it was determined that the most plausible MIE was the ability of TBBPA to inhibit SULT1E1, the enzyme responsible for sulfation of estradiol. Subsequent key events, including increased concentration of estradiol in uterine tissue, would occur with decreased sulfation, leading to a disruption in estrogen homeostasis, increased expression of estrogen responsive genes, cell proliferation, and hyperplasia. Additional key events considered as contributors to the AOP include generation of reactive quinones from the metabolism of estradiol, followed by DNA damage that could contribute to the development of uterine tumors. Uncertainties associated with human relevance are highlighted by potential strain/species and tissue sensitivities as well as the characterization of a dose-dependent MIE. At high doses (such as those used in the cancer bioassay), the TBBPA metabolic profile is altered, suggesting saturation of sulfation, decreased estradiol sulfation and increased tissue estradiol bioavailability. The MIE and subsequent key events for the development of TBBPA induced uterine tumors were determined to be plausible in humans however, are unlikely to occur based on differences in the kinetic and dynamic factors between rats and humans when the current human exposure levels of TBBPA are considered.

3408 Omics Approaches to Evaluate Developmental Neurotoxicity of Organophosphorus Flame-Retardants

H. T. Hogberg[2], R. de Cassia da Silveira e Sa[1,2], M. Bouhifd[2], A. Kleensang[2], A. Maertens[2], O. Cemiloglu Ulker[2,3], L. Smirnova[2], L. Zhao[2] and T. Hartung[2]. *[1]Federal University of Paraiba, Paraiba, Brazil; [2]Johns Hopkins University, Baltimore, MD and [3]University of Ankara, Ankara, Turkey.*

Polybrominated flame-retardants (PB-FRs) have been related to several health issues, including perturbation of fetal and child development. Therefore, they are being substituted with organophosphorus FRs (OP-FRs), despite their resemblance to organophosphate pesticides. Not much data exist on hazard assessment for these OP-FRs, although several of them are suspected to cause developmental neurotoxicity (DNT). The lack of information is partly due to the current DNT guidelines that are based entirely on *in vivo* studies that are time consuming, complex, costly and not suitable for the testing of a high number of chemicals. This study aimed to investigate the DNT effects of four OP-FRs (TPP, IPP, IDDP and TCP) relative to one replaced PB-FR (BDE-47) in a rat primary 3D organotypic *in vitro* model using omics approaches. The model was exposed during development from 7 to 21 days *in vitro*; microarray-based transcriptomics and mass spectrometry-based metabolomics measurements were performed. Data were analyzed using pathway analysis software such as Ingenuity Pathways and Integrated Molecular Pathway Level Analysis. Metabolic networks were built using STITCH database. Combined omics data revealed perturbation in pathways involved in glucose and neurotransmitter trans-membrane transportation, e.g. altered expression of glutamate and glucose transporters as well as changes in metabolite levels involved in the metabolism of sugar and glutamate. Moreover, metabolites involved in butanoate metabolism were altered which are known to be involved in inflammation. Transcriptomics data confirmed inflammation after exposure to the FR as the expression of genes involved in immune response and cytokine activity was upregulated. Moreover, genes involved in neurotransmission and synapse activity were down regulated. Interestingly, the obtained data shows that the selected alternative FRs induced stronger transcriptomics and metabolomics changes in our model than the replaced PB-FR. Our study suggests additional testing of the OP-FRs currently in use. Funded by FDA #U01FD004230, CNPq Post-doctoral grant and in collaboration with NTP, NIH

3409 A Multi-Stage Human Embryonic Stem Cell Differentiation Model for Studying the Effects of PBDEs During Neurogenesis

J. F. Robinson, M. J. Gormley, H. Seifikar, C. J. Fernandez and S. J. Fisher. *Department of Obstetrics, Gynecology & Reproductive Sciences, University of California, San Francisco, San Francisco, CA.*

While the ramifications of polybrominated diphenyl ether (PBDE) exposures during human pregnancy remain unknown, in rodent models, PBDE exposures *in utero* cause neurodevelopmental toxicity. Human embryonic stem cells models (hESCs) are used to study the effects of environmental compounds on the successive stages of neuronal development. Therefore, using a multi-stage hESC model of neuronal differentiation, we investigated the dose-dependent effects of two common congeners, BDE-47 and -99. First, we determined the points of vulnerability to PBDEs across five stages in our model by using a combination of assays to assess for cytotoxicity (i.e., cell viability, death, proliferation). These analyses suggested that differentiated cells are more sensitive to PBDEs than their less differentiated cell precursors. Next, we evaluated the concentration-dependent effects of PBDEs on global gene expression of neural precursor cells after 24h of exposure. In total, we observed 452 genes to be differentially expressed (DE) due to BDE-47 exposure (ANOVA, $p<0.005$; FC>1.5). Functional enrichment analyses of DE genes revealed multiple pathways that were disrupted by BDE-47, including oxidative stress, cell cycle, thyroid hormone signaling, steroid metabolism, morphogenesis, and neurodifferentiation. Validation studies via qRT-PCR confirmed altered expression of select targets (e.g., GRIA1, NRCAM, THRA, THRB, DHCR7, HMOX1, NFE2L2, CDK1) with BDE-47 and/or -99. Our results suggested that PBDEs induced neurodevelopmental toxicity. Sensitivity was dependent on the concentration and the developmental stage of exposure. Ongoing studies aim to further elucidate the mechanisms of PBDE-induced neurodevelopmental toxicity. We are also interested in the general applicability of our hESC neuronal differentiation model for screening environmental compounds for effects on human neurogenesis.

3410 Beyond Science and Decisions: Lessons and Outcomes for Alternatives Assessment (AA) for Flame Retardants and Development of Adverse Outcome Pathways (AOPs)

L. T. Haber[6], O. Kroner[6], B. Meek[8], S. Admon[2], L. H. Pottenger[3], H. J. Clewell[7], M. M. Moore[4], R. Gentry[5], R. Schoeny[1] and M. L. Dourson[6]. *[1]Consultant, Washington, DC; [2]ICL-Industrial Products, Haifa, Israel; [3]Olin, Midland, MI; [4]Ramboll ENVIRON, Little Rock, AR; [5]Ramboll ENVIRON, Monroe, LA; [6]TERA Center, University of Cincinnati, Cincinnati, OH; [7]The Hamner Institute, Research Triangle Park, NC and [8]University of Ottawa, Ottawa, ON, Canada.*

The Beyond Science and Decisions workshop series builds on the NAS (2009) report by review of case studies illustrating risk methods. Methods vetted by the panel, along with links to key guidances, are available at www.chemicalriskassessment.org, a tool for selecting dose-response techniques based on problem formulation. At the most recent workshop, several proposed AOPs were reviewed, as was a proposal on AA. A key strength of the AA framework for evaluating flame retardants (FRs) was the incorporation of qualitative estimates of exposure during the service life stage, based on the anticipated level of product contact and measurable potential emissions of FRs from the matrix in which they are incorporated (e.g. plastics, foams, textile). Review of the AA framework for FRs illustrated the importance of problem formulation and transparency in all aspects of methodology. A key lesson from the workshop for AOPs was distinguishing mode of action (MOA) analysis from evaluation of AOPs based on the evolution of the conceptually identical constructs, and noting where the two methods overlap. AOPs describe the chemically-agnostic toxicodynamic key events (KEs) from the molecular initiating event (MIE) to the adverse effect; multiple chemicals/stressors can elicit the same MIE and ensuing pathway. AOPs are modular, consisting of KEs, linked by KE relationships (KERs), as are chemical-specific MOAs. However, the latter includes metabolic KEs and addresses toxicokinetics in species concordance analysis. AOPs focus on response-response relationships (relating the amount of change in one KE to a specific degree of change in the downstream KE), rather than dose-response relationships for individual chemicals. Peer engagement in developing and evaluating AOPs is helpful in integrating AOPs and MOA analysis for specific chemicals and is best informed by expertise from both areas.

3411 Oxidative Biotransformation of Polybrominated Diphenyl Ethers by Polar Bear Liver Microsomes

S. M. Bandiera, L. K. Krieger and A. Szeitz. *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancovuer, BC, Canada.*

Polar bears (*Ursus maritimus*) are top of the Arctic marine food chain and are subject to exposure and bioaccumulation of environmental chemicals of concern such as polybrominated diphenyl ethers (PBDEs), which were widely used as flame-retardants and have since become ubiquitous environmental contaminants. The aim of the present study was to evaluate the *in vitro* oxidative metabolism of 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabrominated diphenyl ether (BDE-99) by polar bear liver microsomes. The identification and quantification of the hydroxy-brominated diphenyl ether metabolites formed were assessed using an ultra-high performance liquid chromatography-tandem mass spectrometry-based method. Incubation of BDE-47 with archived individual liver microsomes, prepared from fifteen polar bears, produced a total of eleven hydroxylated metabolites, eight of which were identified using authentic standards. The major metabolites were 4′-hydroxy-2,2′,4,5′-tetrabromodiphenyl ether and 3′-hydroxy-2,4,4′-tribromodiphenyl ether. Incubation of BDE-99 with polar bear liver microsomes produced a total of eleven hydroxylated metabolites, seven of which were identified using authentic standards. The major metabolites were 2,4,5-tribromophenol and 4-hydroxy-2,2′,3,4′,5-pentabromodiphenyl ether. Among the CYP specific antibodies tested, anti-rat CYP2B was found to be the most active in inhibiting the formation of hydroxylated metabolites of both BDE-47 and BDE-99, indicating that CYP2B was the major CYP enzyme involved in the biotransformation of these two congeners. This study is the first comprehensive analysis of PBDE hepatic metabolism by polar bear liver microsomes. Our findings show that polar bears are capable of forming multiple hydroxy-PBDE metabolites *in vitro* and demonstrate the role of CYP2B in the biotransformation and possibly in the bioaccumulation and toxicity of BDE-47 and BDE-99 in polar bears.

3412 Hepatic Microsomal Metabolism of BDE-47 and BDE-99 by Lesser Snow Geese

L. K. Krieger, A. Szeitz and S. M. Bandiera. *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada.*

Polybrominated diphenyl ethers (PBDEs) are lipid-soluble, man-made chemicals that were used as flame retardants for over 40 years and have been found to be persistent organic pollutants. Migratory birds such as the lesser snow goose (*Chen caerulescens caerulescens*), which nests on the arctic tundra, are subject to exposure to a wide range of environmental contaminants such as PBDEs. The objective of the present study was to assess the *in vitro* oxidative metabolism of two PBDE congeners, namely 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabrominated diphenyl ether (BDE-99), by liver microsomes from lesser snow geese. Hydroxy-brominated diphenyl ether metabolites were identified and quantified using an ultra-high performance liquid chromatography-tandem mass spectrometry-based method. Incubation of BDE-47 with individual liver microsomes, prepared from six male and six female lesser snow geese, produced a total of sixteen hydroxylated metabolites, eight of which were identified using authentic standards. The major metabolites were 3-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (3-OH-BDE-47), 4-hydroxy-2,2′,3,4′-tetrabromodiphenyl ether (4-OH-BDE-42), 4′-hydroxy-2,2′,4,5′-tetrabromodiphenyl ether (4′-OH-BDE-49). Unidentified metabolites included six di-hydroxy- and two mono-hydroxy-tetrabromodiphenyl ethers. Incubation of BDE-99 with lesser snow goose liver microsomes produced a total of 21 hydroxylated metabolites, twelve of which were identified using authentic standards. The major metabolites were 2,4,5-tribromophenol, 3-OH-BDE-47, 4′-OH-BDE-49 and 4-hydroxy-2,2′,3,4′,5-pentabromodiphenyl ether (4-OH-BDE-90). Unidentified metabolites included mono-OH-pentabromodiphenyl ethers, mono-OH-tetrabromodiphenyl ethers and di-OH-tetrabromodiphenyl ethers. This represents the first study of PBDE metabolism by a migratory avian species. The results show that lesser snow geese can biotransform PBDEs to a large number of oxidative metabolites.

3413 Specific Interactions of BDE-47 and Its Hydroxylated Metabolite 6-OH-BDE-47 with the Human ABC Transporter Proteins P-gp and BCRP

S. A. Marchitti[2], C. S. Mazur[4], C. M. Dillingham[3], S. Rawat[3], A. Sharma[1], J. Zastre[1] and J. F. Kenneke[4]. [1]*Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA;* [2]*ORISE Fellow, US EPA, National Exposure Research Laboratory, Athens, GA;* [3]*Student Services Authority Contractor, US EPA, Athens, GA and* [4]*US EPA, National Exposure Research Laboratory, Athens, GA.*

ATP binding cassette (ABC) transporters, including P-glycoprotein (P-gp; MDR1) and breast cancer resistance protein (BCRP) are membrane bound proteins that mediate the cellular efflux of xenobiotics as an important defense against chemical toxins. Exposure to various environmental chemicals including brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) can result in changes in the gene expression of these transport proteins; however, little information exists on how PBDEs and their metabolites interact with ABC transporters. The purpose of the present study was to assess the interaction of BDE-47 and its more toxic hydroxylated metabolite, 6-OH-BDE-47, with P-gp and BCRP using MDR1- and BCRP-expressing membrane vesicles and stably transfected NIH-3T3-MDR1 and MDCK-BCRP cell lines. Results from membrane vesicle assays measuring P-gp- and BCRP-mediated transport of probe substrates demonstrated that 6-OH-BDE-47 is a more potent inhibitor than BDE-47 of P-gp (IC50 = 11.7 μM vs. no measurable inhibition) and BCRP (IC50 = 9.4 μM vs. IC50 = 45.9 μM) transport activity. P-gp- and BCRP-mediated transport of BDE-47 and 6-OH-BDE-47 as potential substrates was not detected in membrane vesicles. Similarly, minimal protection from P-gp and BCRP against these compounds was demonstrated in cytotoxicity assays performed on NIH-3T3-MDR1 and MDCK-BCRP cells exposed to BDE-47 and 6-OH-BDE-47, as compared to control cells. Further, the cellular uptake of radiolabeled (3H) P-gp and BCRP substrates (paclitaxel and prazosin, respectively) was increased in NIH-3T3-MDR1 and MDCK-BCRP cells in the presence of 6-OH-BDE-47 but not BDE-47, as compared to control cells. Collectively, these results suggest that BDE-47 and its metabolite, 6-OH-BDE-47, are not P-gp or BCRP substrates and, in fact, 6-OH-BDE-47 may actually inhibit P-gp and BCRP efflux activity. Implications for the efflux of xenobiotics by ABC transporters and considerations for human exposure and risk assessment are discussed.

3414 Evidence for Promotion of Pancreatic Cancer by Perfluorooctanoic Acid

L. M. Kamendulis and B. A. Hocevar. *Environmental Health, Indiana University, Bloomington, IN.*

Pancreatic cancer is the fourth leading cause of cancer deaths and one of the most lethal forms of cancer diagnosed in the United States. While the exact cause of pancreatic cancer is unknown, established risk factors for pancreatic cancer include smoking, alcohol consumption and pancreatitis; all of which share the ability to generate oxidative stress - a condition known to promote cancer progression. Perfluorooctanoic acid (PFOA), a chemical widely used in consumer and industrial applications, has been shown to induce pancreatic acinar cell tumors in rodents through a yet to be determined mechanism. We have previously shown that exposure of mice to PFOA triggered oxidative stress in the pancreas, which was associated with focal ductal hyperplasia and inflammation. The goal of this study was to investigate if PFOA promotes pancreatic cancer progression using the well-characterized LSL-KRasG12D;Pdx-1Cre mouse model. Starting at four months of age when early lesions are observable, LSL-KRasG12D;Pdx-1Cre mice received water (control), 0.5 mg/kg or 2.5 mg/kg BW/day PFOA by gavage (5 days/week) for two months. Pancreata were processed for histologic examination and immunohistochemical staining for CK19 which identifies ductal cells, as well as early and late pancreatic lesions including pancreatic adenocarcinoma. We found that the CK19+ lesion area was increased in mice treated with PFOA for two months. qPCR analysis of pancreata verified increased expression of both CK19 and the early lesion marker Sox9 in PFOA-treated groups. In addition, mRNA transcripts for the inflammatory mediators TNFα and IL-1α were increased in PFOA-treated mice. Furthermore, a dose-dependent increase in oxidative stress was observed, evidenced by a 2-fold increase in F2α-isoprostane levels in the pancreas of mice exposed to 2.5 mg/kg PFOA, relative to control. Together, these results demonstrate that PFOA leads to expansion of the pancreatic lesion area in the LSL-KRasG12D;Pdx-1 Cre mouse model which is accompanied by oxidative stress and an inflammatory response.

3415 Enantioselective Actions of PCB 95 Atropisomers Toward Ryanodine Receptors (RyRs) and Hippocampal Neurons

W. Feng[1], G. Robin[1], Y. Dong[1], T. Nakano[2] and I. N. Pessah[1]. *[1]Molecular Biosciences, University of California, Davis, CA and [2]Research Center for Environmental Preservation, Osaka University, Osaka, Japan.*

PCBs with unsymmetrical, multiple chlorine and ortho-substitutions may exist in two stable enantiomeric forms. Enantioselectively enrichment of chiral PCBs, 19 out of 209 congeners, has been reportedly found in environmental samples, biological tissues from plants, animals and humans. Although it is highly possible each enantiomer may have differential interactions with biological targets, functional assessments of enantioselective effect of chiral PCBs are limited due to difficulty in enantiomeric separation. PCB 95 (2,2′,3,5′,6-pentachlorobiphenyl), one of the most potent and efficacious PCBs towards modifying RyR Ca2+ channels, is chiral. Rodent exposure studies have provided evidence showing enantiomeric enrichment of PCB 95 and its hydroxylated metabolites in both dams and their offspring. Here we demonstrate efficient separation of PCB 95 atropisomers using HPLC with three sequential chiral columns and assignments of (+) and (-) enantiomers using circular dichroism spectroscopy. Importantly, we demonstrate that the activity PCB 95 atropisomers broadly differ in their potency toward RyR1 channel sensitization. [3H]ryanodine binding analysis indicates that (-)PCB 95 has significantly greater potency toward stabilizing the RyR1 channel open state than (+)PCB 95, whereas racemic PCB 95 has intermediate potency. Consistent with findings from radioligand binding analysis, single channel voltage clamp studies demonstrate the same rank order of potency and efficacy, with a threshold of 50 pM for (-)PCB 95 enhanced channel activity. Stereoselectivity extends to altering Ca2+ dynamics elicited in murine hippocampal neurons challenged acutely at 14 DIV or subchronically (2-14 DIV) to PCB 95 preparations. These data provide the first separation of PCB 95 atropisomers and evidence of pronounced stereoselectivity towards RyRs and neuromodulation of Ca2+ signaling. Considering selective enantiomeric enrichment of PCB 95 in biological tissues, our mechanistic findings are likely to have significance to understanding human health risks. [Supported by NIEHS R01 ES014901, R01 ES014901, 4P42 ES04699]

3416 Evaluation of the Spatial Distribution of Hepatic Metals Following PCB126 Exposure with X-Ray Fluorescence Microscopy

W. D. Klaren[1], D. Vine[2], S. Vogt[2] and L. W. Robertson[1]. *[1]Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA and [2]Microscopy and X-ray Science Division, Advanced Photon Source, Argonne National Laboratory, Argonne, IL.*

Animal studies have shown that exposure to the environmental pollutant and AhR agonist 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) causes alterations in hepatic metals and micronutrients, in particular: copper, zinc, and manganese. Previous investigations into these effects used acid digested liver tissue employing inductively coupled plasma mass spectrometry (ICP-MS) or a similar analytical techniques. Although informative, these techniques lack the spatial distribution of the metals, within the tissue. Studies have shown that the hepatotoxicity of PCB126 is particularly focused in specific areas. This suggests that the greatest degree of metal disruption may be in the region nearest to this localized hepatic injury. X-ray fluorescence microscopy (XFM) is a powerful technique that allows for the spatial distribution of metals to be ascertained on a tissue/cellular level. Male, Sprague-Dawley rats were fed a modified AIN-93G diet for three weeks, to reach micronutrient equilibrium. The rats were then given a single intraperitoneal injection of either tocopherol-stripped soy oil (5mL/kg), 1 µmol/kg PCB126 or 5 µmol/kg PCB126 in soy oil. After two weeks of exposure, the animals were sacrificed and organs collected. Unstained, formalin fixed, paraffin embedded, sections were placed on silicon-nitride grids and dried in a desiccator. XFM was carried out at the Advanced Photon Source at Argonne National Laboratory. Metal gradients between the central vein and portal triad were seen, especially with copper and iron. Interestingly, clusters of high concentrations of zinc were seen which appear to be in the extracellular space. Differences were seen in animals exposed to PCB126. XFM proves to be a dynamic tool for exploring the micronutrient disruption within a tissue and how toxicants, in particular PCB126, can disrupt this distribution. (P42 ES013661)

3417 Evaluating the Role of the Heart-Liver Axis in Polychlorinated Biphenyl-Induced Toxicity

B. Wahlang, J. Perkins, M. Petriello, J. Hoffman and B. Hennig. *UK Superfund Research Center, University of Kentucky, Lexington, KY.*

Purpose: Polychlorinated biphenyl (PCB) exposures have been correlated with diseases related to metabolic syndrome including liver disease and diabetes. Moreover, fatty liver disease and steatohepatitis have recently been identified as risk factors for cardiovascular diseases but the heart-liver axis is understudied, especially in the context of toxicant exposure. The current study aims to investigate the link between liver toxicity and cardiovascular risks after PCB exposure. Methods: Male C57Bl/6 mice (n=10) were fed either an amino acid control diet (CD) or a methionine-choline deficient diet (MCD) during the 12-week study. Mice were exposed to either PCB 126 (4.9 mg/kg) or the PCB mixture, Arcolor1260 (20 mg/kg) and analyzed for liver fibrosis, fat composition, blood pressure (BP) and calorimetry. Results: Mice fed the MCD diet demonstrated decreased bodyweight and lower % fat composition vs. CD-fed mice, consistent with other MCD-liver fibrosis mouse models. Interestingly, PCB126 induced wasting and mortality only in the MCD-fed mice but not in the CD group. Liver fibrosis development during the study was assessed using ultrasound imaging and all MCD groups exhibited hepatic fibrosis and this was worsened with PCB exposure. Glucose tolerance test was performed and the CD+PCB126 group displayed hypoglycemia while the CD+Aroclor1260 and the MCD groups showed a trend towards insulin resistance. The same phenomenon was observed with BP measurements where CD+PCB126 mice were significantly hypotensive while MCD groups had higher BP. Furthermore, MCD-fed mice had lower energy expenditure and movement in calorimetric studies. Conclusion: PCBs either as a single congener or mixture disrupted normal energy homeostasis and altered cardiac parameters. These effects were pronounced in the context of liver fibrosis. The study is relevant to PCB-exposed human cohorts that may already have a compromised liver due to alcohol or diet-induced fatty liver disease and this could subsequently impact the cardiovascular system.

3418 Perinatal Exposure to PCBs Alters Cocaine Behavioral Sensitization and Dopamine Transporter (DAT) Expression in the Striatum and Medial Prefrontal Cortex of Long-Evans Rats

M. M. Miller, J. L. Sprowles, A. E. Meyer, J. N. Voeller, M. A. Calton and H. J. Sable. *Psychology, University of Memphis, Memphis, TN.*

PCB exposure alters brain dopamine (DA) function by directly affecting the expression of the dopamine transporter (DAT). This alteration can lead to changes in the reinforcing properties of psychostimulants that act upon the DAT. Female Long Evans rats were exposed to 0 (control), 3, or 6 mg/kg/day PCBs throughout gestation and lactation. One adult male and female offspring from each litter were tested as adults in a behavioral sensitization paradigm that examined locomotor activity following repeated daily intraperitoneal (IP) injections of cocaine (COC). Each rat was placed in a locomotor testing chamber and given 30 min to habituate. Next, an IP injection was administered followed by 60 min of activity testing. On day 1, the IP injection was saline while on days 2-7 and 14 it was 10.0 mg/kg COC, and on day 21 it was 20.0 mg/kg COC. No PCB-related differences were observed following the first injection of COC. Behavioral sensitization in the 6 mg/kg/day PCB rats occurred after just the second COC injection which was earlier then in the control group. Significant differences between the PCB-exposure groups were seen following the second COC injection in males, and third COC injection in females. All exposure groups exhibited similar COC behavioral sensitization by the fifth COC injection. At the conclusion of sensitization testing, the medial prefrontal cortex (mPFC) and striatum were extracted for Western blot analysis to determine DAT expression. Results demonstrated that PCB-exposed rats had more DAT expression in mPFC but less in striatum in comparison to control group. Overall, the results indicate that perinatal PCB-exposure can increase the locomotor-activating effects of COC and promote earlier behavioral sensitization to COC perhaps by altering DAT expression in mPFC and striatum. (Supported by NIEHS R00ES015428.)

3419 Serum Concentrations of Non-Ortho Polychlorinated Biphenyls (PCBs) in a Residential Cohort from Anniston, Alabama

M. Pavuk[1], N. D. Dutton[5], A. Sjodin[3], R. S. Jones[3], M. D. Lewin[2] and L. S. Birnbaum[4]. [1]*Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA;* [2]*ATSDR, Atlanta, GA;* [3]*NCEH/CDC, Atlanta, GA;* [4]*NCI, Research Triangle Park, NC and* [5]*ORISE, Atlanta, GA.*

Anniston Community Health Survey: Dioxins and Follow up Analyses (ACHS II) is the second examination of the cohort of Anniston residents living in the vicinity of the former PCB manufacturing facility. While ortho-substituted PCBs have been studied extensively in this population, there is little data on non-ortho PCBs and dioxin congeners. Although the non-ortho PCBs were not major components of production-grade Aroclors, we cannot exclude the possibility of exposure to non-ortho PCB congeners present in PCB-containing waste and refuse. In a 2007 pilot study, we found elevated levels of non-ortho PCB congeners 126 and 169 in a small sample of Anniston residents, which provided the impetus for current analyses. In 2014, 354 people were interviewed and provided blood samples for extensive analytical work, including measurement of non-ortho PCBs 126 and 169. Median age of participants was 63 years; the participants were 52% African-American and 48% White. Measurements using HR GC/MS were reported for 345 samples that satisfied quality control/assurance procedures. Of the 345 samples, 78% had concentrations above the limit of detection for PCB 126 and 88% for PCB 169. The 2014 Anniston residents' median concentrations were 24.71 pg/g lipid for PCB 126 and 26.81 pg/g lipid for PCB 169. These results are similar to medians for the >60-year-old group from US general population in 2003-2004 (NHANES 2003-2004, 31.50 and 25.90 pg/g lipid, respectively). However, the 75th percentile concentrations of both congeners (PCB 126: 69.35 pg/g lipid, PCB 169: 44.78 pg/g lipid) were above the 75th percentile of NHANES 2003-2004 (50.7 and 37.2 pg/g lipid, respectively). Preliminary analyses do not suggest elevations in dioxin or dibenzofuran congeners, but age- and race-specific groups have yet to be examined in detail. Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by [the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry] and should not be construed to represent any agency determination or policy.

3420 PCB126-Induced Disruption in Gluconeogenesis and Fatty Acid Oxidation Precedes Fatty Liver in Male Rats

G. S. Gadupudi[3], W. D. Klaren[3], A. K. Oliver[4], A. J. Klingelhutz[3] and L. W. Robertson[3]. [1]*Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA;* [2]*Microbiology, University of Iowa, Iowa City, IA;* [3]*Occupational and Environmental Health, University of Iowa, Iowa City, IA and* [4]*Pathobiology and Population Medicine, Mississippi State University, Starkville, MS.*

3,3',4,4',5-Pentachlorbiphenyl (PCB126), a potent aryl hydrocarbon receptor (AhR) agonist, is implicated in the disruption of both carbohydrate and lipid metabolism which leads to metabolic disease and non-alcoholic fatty liver disease (NAFLD). However, the mechanisms are unclear. Hence, we hypothesized that early disruption of glucose and lipid homeostasis contributes to later manifestations such as hepatic steatosis. To test this hypothesis, groups of male Sprague-Dawley rats, fed an AIN-93G diet, were injected (i.p) with a single bolus of PCB126 (5 µmol/kg) at various time intervals between 9 h and 12 days prior to euthanasia. An early decrease in serum glucose and a gradual decrease in serum triglycerides were observed over the time course. Liver lipid accumulation was most severe at 6 and 12 days post exposure. Transcript levels of cytosolic phosphoenol-pyruvate carboxykinase (Pck1) and glucose transporter (Slc2a2) involved in gluconeogenesis and hepatic glucose transport, were time-dependently downregulated between 9 h and 12 d of PCB126 exposure. Additionally, transcript levels of Pparα, and its targets acyl-CoA oxidase (Acox1) and hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2), were also downregulated, indicating changes in peroxisomal fatty acid oxidation and ketogenesis. In a separate animal study, we confirmed that changes in the transcript levels of Pepck-c, Glut2, Pparα, Acox1 and Hmgcs2 were also dose-dependent. Further, PCB126-induced effects on Pepck-c were demonstrated to be AhR dependent in rat H4IIE hepatocytes. These results indicate that PCB126 induced wasting and steatosis are preceded initially by a) decreased serum glucose caused by decreased hepatic glucose production, followed by b) decreased peroxisomal fatty acid oxidation.

3421 Distinct Metabolomic Axes in Gulf Coast Minnows After Deepwater Horizon Oil Spill

J. D. Chandler[1], A. Todor[1], J. Wise[2], D. P. Jones[1], R. J. Griffitt[2] and S. Li[1]. [1]*Emory University, Atlanta, GA and* [2]*University of Southern Mississippi, Ocean Springs, MS.*

High-resolution mass spectrometry (MS) has become a powerful tool to measure the metabolome and exposome in biological samples. We used an untargeted LC-MS platform (Thermo Scientific Q-Exactive hybrid Orbitrap MS coupled with reverse-phase C18 lipid chromatography) to profile Sheepshead minnows (Cyprinodon variegatus) caught from the Gulf of Mexico in 2013 after the Deepwater Horizon oil spill disaster and compared these to liver samples from lab-raised individuals. We measured 9,843 chemical features in the minnows livers (N = 40), and 6,688 were used for subsequent analysis after quality filtering. We next tested the association of these chemical features to the gene expression levels of two target genes of the aryl hydrocarbon receptor (AhR), CYP1A1 and VEGF, in these samples. Both AhR target genes were increased in expression in wild-caught minnows, suggesting persistent organic pollutant exposure in the wild individuals. Chemical features associated with CYP1A1 were highly enriched for a large number of metabolic pathways, including metabolism of glutamate, tryptophan, tyrosine and ascorbate. VEGF expression was associated with pantothenate and xenobiotic metabolism pathways. This study demonstrates broad hepatic metabolite disruption corresponding to probable pollutant exposure and increased AhR activity in wild caught Sheepshead minnows.

3422 Sex Differences in Dichlorodiphenyltrichloroethane (DDT) and Dichlorodiphenyldichoroethylene (DDE) Potency Towards Ryanodine Receptor Type 2

K. M. Truong and I. N. Pessah. *Molecular Biosciences, UC Davis, Davis, CA.*

Legacy pollutants dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichoroethylene (DDE) are ubiquitously found in the environment and are known to be endrocrine disruptors. While the estrogenic activity of DDT and DDE have been widely studied, less is known about their effects on cardiac muscle, which is subject to both electrical and hormonal regulation. Since males and females differ in hormonal levels and it is known that DDT and DDE affect estrogen receptors, our study aimed to determine whether there are sex differences in the potencies of DDT and DDE towards the ryanodine receptor type 2 (RyR2), a protein necessary for physiological cardiac excitation-contraction coupling and pathophysiological sequelae leading to heart disease. We generated cardiac muscle sarcoplasmic reticulum (SR) preparations from whole hearts of individual male and female C57BL/6J wild-type mice. These SR preparations were subjected to radioligand-receptor binding analysis with [3H]ryanodine (5 nM) in the presence of either vehicle (DMSO) or 10 µM of pure DDT or DDE congener. Both DDE congeners and o,p'-DDT significantly activated RyR2 from male cardiac muscle whereas only o,p'-DDT and o,p'-DDE were able to significantly activate RyR2 from female cardiac muscle. Three of the four compounds tested (o,p'-DDT, p,p'-DDT, and o,p'-DDE) had significantly stronger effects in male versus female preparations, whereas the fourth (p,p'-DDE) exhibited similar activity. These results suggest sex differences in the cardiac responses to these pollutants: males are more susceptible to the activating effects of DDT and DDE than females, perhaps due to differences in hormonal regulation between the two sexes. There is a need to better understand how fluctuations in hormone levels influence post-translational modifications of RyR2 and their influences on responsiveness to DDT and DDE-congeners, and whose dysregulation can lead to impairments such as heart failure. [Supported by NIEHS R01 ES014901, 4P42 ES04699]

3423 Transcriptional and Post-Transcriptional Regulations of Acetylcholinesterase Expression by Dioxins

T. Xu, R. Sha, Z. Guo, H. Xu, S. L. Xu, H. Q. Xie and B. Zhao. *Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China.*

Acetylcholinesterase (AChE, EC3.1.1.7) plays an important role in cholinergic neurotransmission, which is also a well-known biomarker for monitoring pesticides pollutions. Emerging evidence showed that the enzymatic activity of AChE was altered by more and more xenobiotics, including heavy metals, nano-particles, persistent organic pollutants. We previously reported that dioxin treatment at 0.1 to 1 nM significantly suppressed the enzymatic activity of AChE in human neuroblastoma

cells. However, the transcriptional suppression via aryl hydrocarbon receptor was not able to fully explain the underneath mechanism for the effects on AChE. In addition to transcriptional mechanism, post-transcriptional regulation involving microRNA (miR) needs to be examined, since TCDD was able to affect the expression of miRs in various cell types. Therefore, in the present study, we performed miRs microarray analysis using the human neuroblastoma cells treated with dioxin at 0.1 nM. As compared to solvent control, totally 233 differentially expressed miRs ($p \leq 0.05$) were found in TCDD-treated cells by Robust Multichip Average algorithm. Among them, 17 miRs have been predicted as AChE-targeting miRs in literature, which might serve as candidates mediating post-transcriptional regulation of AChE expression by dioxins.

3424 Human Health Concerns of the Levels of Polyaromatic Hydrocarbons (PAHs) Congeners in E-Wastes Contaminated Sediments from Tropical Coastal Ecosystems

J. K. Igbo[2], O. L. Chukwu[4], E. O. Oyewo[1] and J. T. Zelikoff[3]. [1]*Biological Oceanography, Nigeria Institute for Oceanography and Marine Research, Vl, Lagos, Nigeria;* [2]*Biological Oceanography, Nigerian Institute for Oceanography and Marine Research, Vl, Lagos, Nigeria;* [3]*Environmental Medicine, New York University, New York, NY and* [4]*Marine Sciences, University of Lagos, Lagos, Nigeria.*

The environmental, ecological as well as human health risks associated with discharges of e-waste or seepage of the leachates from open waste dumpsites into ground water and aquatic ecosystems especially in developing economies calls for concern. This study assessed the levels of congener profiles of priority 16 PAHs in sediment from two e-waste contaminated coastal ecosystems (Southwest Nigeria) by Gas-chromatographic analyses using GC-FID Agilent 7890 A. The results pointed out a non-homogenous contamination of the two aquatic ecosystems with high molecular weight (HMW) (6 rings) congeners with values ranging from 158.77µg/kg (Lagos) to 81.30µg/kg (Osun), reflecting moderate to low contamination probably due to particular hydrological characteristics of the sites and/or non-homologous inputs from point sources (electronic wastes from the dump sites) of these compounds. The general order of decreasing congener contribution to the total load was: 6 rings >3 rings> 5 rings> 4 rings> and 2 rings. Although no uniform temporal and seasonal trend of PAHs was recorded, predominantly higher levels of congeners (78%) were recorded in the wet season. The results from the present study shows that the indiscriminate and uncontrolled dumping of e-wastes in developing countries needs to be addressed urgently considering the potential risks associated with the accumulation in wildlife and human food webs in these ecosystems and attendant health implications .

3425 Toxicity Evaluation of Exposure to an Atmospheric Mixture of Polychlorinated Biphenyls by Nose-Only and Whole-Body Inhalation Regimens

X. Hu[2], A. Adamcakova-Dodd[2], H.-J. Lehmler[2], K. N. Gibson-Corley[2] and P. S. Thorne[2]. [1]*Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA and* [2]*Occupational and Environmental Health, University of Iowa, Iowa City, IA;* [3]*Pathology, University of Iowa, Iowa City, IA.*

The health risk of inhalation exposure to polychlorinated biphenyls (PCB) cannot be assessed with high confidence due to the lack of rigorous inhalation studies. One uncertainty rests on exposure regimen, as whole-body exposure systems allow oral PCB intake that confounds the exposure. We conducted contemporaneous PCB inhalation exposures with whole-body and nose-only exposure methods to better inform risk assessment for inhaled PCBs. Female Sprague-Dawley rats were concurrently exposed to vapor-phase PCBs (533 ± 93 µg/m3) generated from PCB11-supplemented Chicago Air Mixture resembling the Chicago airshed, 4 h/day, 6 days/week, for 4 weeks. Congener-specific analysis showed 1.5-fold higher ΣPCBs in the lungs of nose-only exposed than the whole-body exposed animals ($p = 0.0024$). Higher ΣPCB concentrations were also found in the sera, livers, brains, and adipose tissue of nose-only exposed animals (1.1-1.5-fold), but these increases were not statistically significant. Congener profiles of five tissue types were dominated by PCB 28/31 and higher-chlorinated congeners in both groups reflecting rapid metabolism of other lower-chlorinated PCBs. No toxicity was seen regarding metabolic enzyme expression, glutathione, or histopathology. However, diminished weight gain and reduced plasma total thyroxine levels were found in both groups compared with controls, after exposure to 76 µg/m3 ΣPCBs as adjusted for continuous exposure. Hepatic lipid peroxidation was also elevated in the nose-only group. Our study identified a low dose adverse effect level and shows that prolonged nose-only exposure was well-tolerated and eliminated the need for housing animals outside the vivarium, thus was preferred for long-term PCB inhalation studies. Supported by NIEHS through a supplement to NIH P42 ES013661 & NIH P30 ES005605.

3426 Exposure to Organochlorine Pesticide Metabolites Increase *De Novo* Lipogenesis and Free Fatty Acid Accumulation to Promote Hepatic Steatosis *In Vitro*

G. E. Howell, C. Mulligan and W. Gordon. *College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.*

A common pathological alteration in type 2 diabetes is the development of non-alcoholic fatty liver disease (NAFLD) or hepatic steatosis. Recent studies have revealed significant associations between exposures to various pesticides or their metabolites, including organochlorine pesticides, and the occurrence of type 2 diabetes. Given these associations, the present study was designed to determine if direct exposure to the highly implicated organochlorine (OC) pesticide metabolites p,p'-dichlorodiphenyldichloroethylene (DDE), trans-nonachlor, and oxychlordane alter hepatocyte lipid metabolism to promote neutral lipid accumulation and steatosis. The effect of exposure to OC pesticide metabolites on hepatocyte neutral lipid accumulation was determined in both McArdle-RH7777 (McA) rat hepatoma cells and rat primary hepatocytes. Exposure to trans-nonachlor and oxychlordane produced concentration dependent increases in neutral lipid accumulation in McA cells. However, only trans-nonachlor significantly increased neutral lipid accumulation in primary hepatocytes. The increased neutral lipid accumulation in McA cells is due, at least in part, to increased intracellular triglyceride levels. To determine possible mechanisms governing increased fatty acid availability for triglyceride formation, the effects of OC exposure on fatty acid uptake and *de novo* lipogenesis were explored. Exposure to trans-nonachlor or oxychlordane for 24 hours significantly increased fluorescently labeled free fatty acid accumulation that does not appear to be due to increased fatty acid uptake. To assess potential effects on lipogenesis, cellular levels of the key lipogenic proteins fatty acid synthase (Fas) and acetyl-CoA carboxylase-1 (Acc-1) were determined in primary hepatocytes following exposure to trans-nonachlor or oxychlordane for 24 hours. Exposure to trans-nonachlor and oxychlordane significantly increased cellular levels of Fas and Acc-1. Taken together, the present data indicate direct exposure to the OC pesticide metabolites trans-nonachlor and oxychlordane can alter hepatocyte lipid metabolism including increased *de novo* lipogenesis and increased free fatty acid accumulation to promote hepatocyte neutral lipid retention which is a hallmark of hepatic steatosis.

3427 Low-Doses Dioxin Subchronic Exposure Promotes Liver Fibrosis Development in Diet-Induced Obese C57BL/6J Mice

C. Duval[2,6], F. Teixeira-Clerc[1,8], A. Leblanc[2,6], M. Guerre-Millo[4,9], C. Emond[5], S. Lotersztajn[3,7], R. Barouki[2,6], M. Aggerbeck[2,6] and X. Coumoul[2,6]. [1]*INSERM U955, Paris, France;* [2]*INSERM UMR-S 1124, Paris, France;* [3]*INSERM UMR-S 1149, Paris, France;* [4]*INSERM UMR-S 872, Paris, France;* [5]*Montréal University, Montréal, QC, Canada;* [6]*Paris Descartes University, Paris, France;* [7]*Paris Diderot University, Paris, France;* [8]*Paris-Est University, Paris, France and* [9]*Pierre et Marie Curie University, Paris, France.*

Exposure to persistent organic pollutants (POP) has been associated with the progression of chronic liver diseases, yet the potential contribution of POPs to the development of fibrosis in non-alcoholic fatty liver disease (NAFLD), a condition closely linked to the obesity epidemic, remains poorly documented. Our aim was to study the effect of subchronic exposure to low-doses of the POP 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a ligand of the aryl hydrocarbon receptor, on NAFLD progression in the C57BL/6J diet-induced obesity mouse model. We first established with a dose-response curve that 5 µg/kg TCDD is the threshold dose for liver collagen deposition after subchronic administration in mice. Then, C57BL/6J male mice were either fed a 10% low fat or a 45% high fat diet (HFD) during 14 weeks and injected once a week with 5 µg/kg TCDD or vehicle for the last 6 weeks of the diet intervention. TCDD exposure, regardless of the diet, did not impair adiposity nor plasma leptin and insulin levels. By contrast, liver histology and triglyceride quantification revealed that co-exposure to HFD and TCDD worsens hepatic steatosis compared to HFD or TCDD alone. Accordingly, hepatic mRNA levels of key genes (Cd36, Cpt1a, Fas, Dgat2, Mttp) as well as crucial modulators (Ppara, Pparg, Srebf1, Chrebp) of lipid metabolism were strongly altered in co-treated mice. More importantly, TCDD induces liver fibrosis in HFD fed mice, as shown by increased liver collagen stain-

ing and serum transaminases levels. However, although TCDD increased gene expression of inflammatory (Ccl2, Il1b, Tgfb1) and fibrotic (Acta2, Col1a1, Col3a1) markers, there was no additive effect upon HFD, suggesting that other molecular targets underlie collagen accumulation. In conclusion, the combination of exposure to TCDD together with an enriched fat diet amplifies the risk of impairment of liver functions in mice. Our data provide new evidence that environmental pollutants could promote liver fibrosis development in obesity-related NAFLD.

3428 Determining the Role of the Gut Microbiome in Altering Drug Metabolism

M. A. Malfatti, E. A. Kuhn, N. Hum, D. K. Murugesh, C. J. Jaing and G. G. Loots. *Biosciences and Biotechnology, Lawrence Livermore National Laboratory, Livermore, CA.*

The gut microbiota are associated with essential various biological functions through a network of microbial – host co-metabolism to process nutrients and drugs. Variation in gastrointestinal (GI) metabolism is related directly to the activities of the various microorganisms that coexist in the gut. Shifts in the composition of GI microbiota, whether induced by dietary changes, antibiotic treatment or invasive pathogens can disturb the balance of organisms in the gut and alter the metabolic networks of the host. Changes in the microbiome can also influence the overt toxicity and metabolism of drugs. To determine how changes in the gut microbiome can alter drug metabolism and affect drug interactions, the pharmacokinetics (PK) and biodistribution of the model compound acetaminophen (APAP) was assessed in C57/Bl6 mice after exposure to lipopolysaccharide (LPS), to induce an inflammatory response, followed by treatment with the antibiotic Ciprofloxacin (Cipro). Altered tissue concentration, plasma PK and metabolism of APAP was observed over 24 h, in animals exposed to LPS followed by Cipro. Plasma Tmax and AUC were significantly increased over control animals and the maximum concentration of APAP in the stomach and kidneys were delayed in the LPS/Cipro treated animals compared to the other treatment groups suggesting that APAP persists in the gut, and tissues longer in co-administered drug regimes. Urinary metabolite profiles revealed changes in APAP metabolite levels in LPS/Cipro treated animals compared to control animals. Analysis of gut microbe composition using the LLNL Microbial Detection Array system revealed changes in microbe content in LPS/Cipro treated animals compared to untreated animals. These results suggest that exposure to LPS and/or Cipro can alter the biodisposition of APAP and that these alterations could be directly due to changes in gut microbiome composition. This work was performed under the auspices of the US DOE by LLNL under Contract DE-AC52-07NA27344 and supported by the National Institute of General Medical Sciences (P41 GM103483-14).

3429 Role of Intestinal Microbiota on Pharmacokinetics and Toxicity of Baicalin in Mice

M. J. Kang, M. J. Kong, K. Noh, M. R. Nepal, K. S. Jeong, J. Ahn and T. C. Jeong. *College of Pharmacy, Yeungnam University, Gyeongsan, Korea, Republic of.*

Intestinal microbiota plays an important role on the drug metabolism in intestine, and affects the pharmacokinetics of drugs. Baicalin is a representative example which is metabolized by the intestinal microbiota. To demonstrate the effect of intestinal microbiota on the pharmacokinetics of baicalin, 100 mg/kg baicalin was orally administered to SPF mice treated with and without an antibiotic mixture of cefadroxil, oxytetracycline, and erythromycin, as well as to germ-free mice. And then, blood and tissues (i.e., liver, kidney, and lung) were collected to evaluate baicalin pharmacokinetic and histopathological changes. As a result, the systemic exposure of baicalin was reduced, and pharmacokinetic parameters were also remarkably changed in antibiotic-treated and germ free mice without any histopathological changes in tissues. Furthermore, AST and ALT were significantly increased following baicalin administration in germ-free mice than control mice. The results suggested that the intestinal microbiota were responsible for pharmacokinetic change of baicalin, and the microbiota had a hepatoprotective effect against liver toxicity induced by baicalin *in vivo*.

3430 The Anti-Oxidant Drug Tempol Promotes Functional Metabolic Changes in the Gut Microbiota

J. Cai[2], L. Zhang[2], R. A. Jones[2], J. B. Correll[2], E. Hatzakis[2], P. B. Smith[2], F. J. Gonzalez[1] and A. D. Patterson[2]. [1]*NIH, Bethesda, MD and* [2]*Penn State University, University Park, PA.* Sponsor: A. Patterson.

Trillions of microbes reside in the gastrointestinal tract and can significantly modulate host metabolism. Further the gut microbiota are important determinants of the response to xenobiotics including drugs, environmental contaminants, and dietary chemicals. Tempol, a stable free radical nitroxide, is known to cause weight loss in rodent models and improves many obesity-related complications such as non-alcoholic fatty liver disease. However the concrete mechanism for these beneficial effects of tempol remain unclear. To investigate the metabolic changes associated with tempol treatment, conventional and germ free mice were orally administered tempol for five days. Metabolites from mouse cecal contents, feces, and liver were analyzed by a combination of GC-MS (Agilent 7980A GC/5950MSD) and 1H NMR (Bruker Avance-III 600 MHz). A dose-dependent decrease in bacterial fermentation was found in tempol-treated mice, indicated by significantly lower levels of short chain fatty acids (acetate, butyrate, and propionate) in cecal and fecal extracts as well as increased excretion of fecal oligosaccharides. Bomb calorimetry of fecal pellets confirmed these results finding that tempol-treated mice had greater energy excretion compared to vehicle-treated mice. Moreover, liver 1H NMR-based metabolomics identified a dose-dependent decrease in glycogen and glucose, enhanced glucogenic and ketogenic activity (tyrosine and phenylalanine), and increased activation of the glycolysis pathway. Serum 1H NMR-based metabolomics indicated tempol promotes enhanced glucose catabolism. Hepatic gene expression was significantly altered as demonstrated by an increase in Pepck and a decrease in Hnf4a, ChREBP, Fabp1, Fabp5, and Cd36. This study demonstrated that tempol treatment decreases host energy availability by inhibiting microbial fermentation. Additionally, tempol treatment caused a pronounced shift in liver metabolism towards catabolic pathways (e.g., glycogenolysis, lipolysis, glycolysis) to compensate for the insufficient energy availability. The study suggests a possible mechanism for the observed anti-obesity effect of tempol and demonstrate clearly that xenobiotics can impact host metabolism.

3431 Post-Transcriptional Regulation of the ABC Transporters Mrp2, Bcrp and P-gp in Enterocytes During Acute Acetaminophen (APAP) Intoxication in Mice

C. I. Ghanem[1,5], L. Orbea[5], S. Rudraiah[3], A. D. Mottino[4] and J. E. Manautou[2]. [1]*Cátedra de Fisiopatología., Facultad de Farmacia y Bioquímica. UBA., Buenos Aires, Argentina;* [2]*Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT;* [3]*Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT;* [4]*Instituto de Fisiología Experimental (IFISE), CONICET-UNR, Buenos Aires, Argentina and* [5]*Instituto de Investigaciones Farmacológicas (ININFA), CONICET-UBA, Buenos Aires, Argentina.*

We have previously described the transcriptional regulation of ABC transporters in liver and brain in reponse to APAP treatment. The aim of the present work was to study the apical ABC transporters expression in the small intestine (Mrp2, Bcrp and P-gp) during APAP intoxication. For this purpose, male C57BL6 mice were given APAP (400mg/kg, i.p.) or vehicle. All sections of the small intestine were removed at 6, 12, 24 or 48 h after treatment. At 24 and 48 h, intestinal Mrp2, Bcrp and P-gp protein expression was determined by Western blotting using brush border membrane only. mRNA expression for these ABC transporters was also assayed by qRT-PCR at 6, 12 and 24 h. Immunohistochemical analysis of Mrp2, Bcrp and P-gp was also performed. Protein expression of Mrp2, Bcrp and P-gp was significantly increased in the proximal region of the intestine of APAP treated mice by 153%; 208% and 149%, respectively. By 48 h, Mrp2, Bcrp and P-gp protein expression returned to basal values, which is consistent with immunohistochemical analysis as well. Interestingly, the mRNA expression of any of these ABC transporters was not different between APAP and vehicle groups at any of the time points examined. In conclusion, our data demonstrates that during APAP intoxication in mice, the protein expression of the apical ABC transporters (Mrp2, Bcrp and P-gp) was up-regulated in the intestine of mice. The lack of changes in mRNA expression of these transporters is indicative of a post-transcriptional regulation mechanism that is as yet unresolved.

3432 Organic Anion Transporting Polypeptides Mediated Drug Interaction Between Rosuvastatin and Cyclosporin A in Chimeric Mice with Humanized Liver

Y. Tajima[1], M. Uchida[1], M. Kakuni[2], Y. Kageyama[2], T. Okada[2], C. Tateno[2] and R. Hayashi[1]. [1]*Pharmaceutical Research Laboratories, Toray Industries, Inc., Kamakura, Japan and* [2]*Study Service Department, Phoenixbio Co., Hiroshima, Japan.* Sponsor: K. Oshida.

Cyclosporin (CsA), known as OATP inhibitor, has reported to increase the AUC of rosuvastatin (RS), known as OATP substrate, by 7.1-fold in heart transplant recipients. PXB-mice, chimeric animal models with humanized liver, are urokinase-type plasminogen activator/severe combined immunodeficiency (uPA/SCID) mice repopulated with 80% or more human hepatocytes. It has been reported that a lot of human transporters and metabolic enzymes are expressed in the liver of PXB-mice. We examined OATPs-mediated drug interaction between RS and CsA in PXB-mice, compared with SCID mice as control mice. The area under the blood concentration-time curve (AUC0-4h) of intravenously administered RS was increased by 2.5 to 6.2-fold in PXB-mice, when pretreated with CsA, while it was increased by 4.6-fold in SCID mice pretreated with CsA. On the other hand, the AUC0-4h of orally administered RS was increased by 3.3 to 11-fold in PXB-mice, when pretreated with CsA, while was increased by 13-fold in SCID mice pretreated with CsA. The AUC increase in orally administered RS with CsA was slightly higher than that in intravenous administered RS. Liver-to-blood concentration ratios of RS were decreased by the pretreatment with CsA in both PXB- and SCID mice. Moreover, lactone form (human UDP-glucuronosyltransferase 1A metabolite) and N-desmethyl form (human cytochrome P450 2C metabolite), known as human metabolites of RS, were monitored. Lactone form was detected in liver of PXB-mice and N-desmethyl form was also detected in plasma and liver of PXB-mice, while these metabolites were hardly detected in SCID mice. It suggests that these metabolites might be human-specific or -abundant metabolites. Our results indicate that PXB-mice would be useful tools for predicting human OATP-mediated DDIs and human metabolites.

3433 Inter-Individual Variability in the Relationship Between Toxicokinetics and Toxicodynamics of Tetrachloroethylene

J. A. Cichocki, S. Furuya, A. Venkatratnam, G. Chappell, S. Sweet, T. Wade, T. Knap, W. Chiu, D. Threadgill and I. Rusyn. *Texas A&M University, College Station, TX.*

Data on inter-individual variability in susceptibility to the adverse health effects of environmental chemicals are seldom available for risk assessment. Variability in response to chemicals can be mediated by differences in toxicokinetics (TK) or toxicodynamics (TD). The goal of the current study was to investigate the extent of variability in TK and its relationship to TD across a mouse model of the human population (Collaborative Cross; CC) using the ubiquitous environmental pollutant tetrachloroethylene (PERC) as a model toxicant. Toxic metabolites of PERC are proposed to be responsible for PERC toxicity. It was hypothesized that variation in PERC TK as a consequence of genotype would be associated with variation in TD of PERC. To this end, adult male mice from 45 strains of CC were exposed to a single intragastric dose of PERC (1,000 mg/kg) or vehicle (5% Alkamuls-EL620 in saline); tissues were harvested 1-24 hr post dosing. Metabolite profiling revealed that substantial variability in partial areas under the curve (AUCs) for trichloroacetate (TCA), the primary oxidative PERC metabolite, were observed in liver, kidney, lung, brain, fat, and serum. The greatest degree of variability was observed in serum TCA AUCs (range: 5,000-50,000 nmol/mL*hr). As oxidative metabolism of PERC is associated with liver toxicity, this tissue was further examined. Basal levels of hepatic cytochrome P450-2E1 (CYP2E1) were variable across the population, however no statistical correlation was observed between hepatic CYP2E1 and TCA levels, suggesting that multiple enzymes contribute to the oxidation of PERC. Induction of genes regulated by peroxisome proliferator-activated receptor-alpha (PPARα) was observed in the livers of most strains; the effect was highly variable (range of fold-induction: 1-200). TCA can activate PPARα, however no statistical correlation was observed between hepatic TCA AUCs and induction of PPARα-responsive genes. Taken together, these results demonstrate that the Collaborative Cross is a useful resource for exploiting inter-individual variability in toxicokinetics and toxicodynamics of environmental chemicals. This work shows that additional characterization of the mechanisms of inter-individual variability is needed to better assess human health risks associated with exposure to PERC.

3434 Using the Collaborative Cross Mouse Model to Investigate Population-Level Variability in Trichloroethylene Toxicity

A. Venkatratnam[2,5], S. Furuya[5], O. Kosyk[2], V. Soldatow[2], S. Sweet[5], T. Wade[5], A. Knap[5], A. Gold[2], W. Bodnar[2], W. Chiu[5] and I. Rusyn[5]. [1]*Texas A&M, College Station, TX;* [2]*Environmental Sciences of Engineering, University of North Carolina, Chapel Hill, NC;* [3]*Geo Chemical and Environmental Research Group, Texas A&M, College Station, TX;* [4]*The Department of Veterinary Integrative Biosciences, Texas A&M, College Station, TX and* [5]*Veterinary Integrative Bio Sciences, Texas A&M, College Station, TX.*

Trichloroethylene (TCE) is an environmental pollutant and a human carcinogen, but there is a critical need to better understand inter-individual differences in TCE susceptibility. Previous studies have evaluated inter-strain variability in TCE toxicokinetics/toxicodynamics (TK/TD) using liver and serum derived data acquired from studies in multi-strain mouse model. This study aims to further improve assessment of inter-individual variability in TCE in three ways: using a genetically-defined and reproducible mouse population, the Collaborative Cross (CC); measuring TK/TD markers in additional tissues; and including both sexes. We hypothesized that genetic variability among CC population will aid in characterizing inter-individual differences in TCE responses. In one experiment, adult male mice from 50 CC strains were exposed to a single oral dose of TCE (24, 80, 240 or 800 mg/kg) and tissues were collected and evaluated at 24 hr. In another experiment, males and females from 5 CC strains were exposed to a single oral dose of TCE (800 mg/kg) and tissues were collected and evaluated at 1-36 hr post dosing. In the TD study, we observed substantial induction of hepatic PPAR-α responsive genes in the highest dose group with up to 100-fold differences among the 50 CC lines. Although there was up to 40-fold inter-individual variability in the 24 hr hepatic levels of TCA, a major oxidative pathway metabolite of TCE and peroxisome proliferator, no correlation with PPAR-α responsive gene transcript levels was observed. Further, no association between basal or post-treatment hepatic CYP2E1 protein levels and TCA levels was found. In the TK study, inter-strain variability in TCA AUC was similar to that in 24-hr TCA levels from the first study across several tissues. Additionally, hepatic TCA AUCs was found to be 2.5-fold higher in males across strains. These results demonstrate the utility of the CC model in characterizing the magnitude of inter-individual variability in toxicant-induced effects.

3435 *In Vivo* Skin Absorption of Ortho-Phenylphenol in Metalworking Fluid Formulations

E. Nixon, J. Brooks and R. Baynes. *North Carolina State University, Raleigh, NC.*

Every year, ten million workers are exposed to metalworking fluids (MWFs) which may be toxic, especially to the respiratory system and the skin. There are four types of MWFs; neat oils and three water based MWFs (soluble oil, semi-synthetic and synthetic) that vary in composition of performance additives. An example of one of these additives is the biocide, ortho-phenylphenol (OPP). The objective of this study was to assess the skin absorption of C14-OPP in MWF formulations (water only and a generic synthetic MWF formulation) in pigs (n = 4) *in vivo* following a 6-day skin exposure to 1250 µg OPP. Pigs (n = 4) were also dosed intravenously (IV) to obtain pharmacokinetic parameters. Blood, feces, urine and tissue samples were collected from all pigs for analysis in a liquid scintillation counter. Pharmacokinetic analyses of the data involved use of compartmental and non-compartment models. The half-life of C14-OPP (IV bolus) was 23.67 ± 5.63 h which was comparable to that in humans. C14-OPP had greater skin absorption and reached a higher concentration in the blood from a topical water only dose (AUC = 3,094 ± 1,466 h*ng/mL, Cmax = 45.34 ± 8.91 ng/mL) compared to the topical synthetic formulation (AUC = 1,159 ± 668 h*ng/mL, Cmax = 20.79 ± 13.83 ng/mL). The kidneys played a crucial role in clearance of C-14 OPP with a large percentage of the dose being found in the urine with the IV bolus dose (70.6 ± 6.9 %dose). Clearance/F was greater from the synthetic formulation (48.7 ± 33.1 mL/h/kg) compared to the water only dose (21.1 ± 12.7 mL/h/kg). Absolute skin bioavailability was 15.24% for the water-only formulation and 8.04% for the synthetic MWF. Our previous *in vitro* study demonstrated a comparable (3 - fold) difference in absorption. OPP is a lipophilic compound and the data supports this as almost twice as much OPP was available systemically with the water only formulation than with the synthetic MWF formulation. Current research will elaborate on these findings with semi-synthetic and soluble oil formulations of MWF. With this data, the MWF industry would be able to adjust their MWF formulation to protect the metalworkers' health.

3436 Assessment of *In Vitro* High-Throughput Pharmacokinetic Data to Predict *In Vivo* Pharmacokinetic Data of Environmental Chemicals

M. F. Hughes[3], C. L. Ring[1], T. R. Fennell[2], D. K. MacMillan[3], J. E. Simmons[3], R. S. Thomas[3] and J. F. Wambaugh[3]. *[1]ORISE, Research Triangle Park, NC; [2]RTI, Research Triangle Park, NC and [3]US EPA, Research Triangle Park, NC.*

Assessing the health risks of the thousands of chemicals in use requires both toxicology and pharmacokinetic (PK) data that can be generated more quickly. For PK, *in vitro* clearance assays with hepatocytes and serum protein binding assays provide a means to generate high throughput (HT) PK data. A PK model is used to extrapolate the *in vitro* data to *in vivo* exposures. In this study, we assessed the ability of current *in vitro* HTPK models to accurately predict *in vivo* PK parameters, such as peak plasma concentrations (Cmax), volume of distribution (Vd), and half-life. Twenty-six ToxCast chemicals were evaluated, including a variety of pesticides (e.g., carbofuran) and industrial chemicals (e.g., bisphenol A). Adult male Sprague-Dawley rats with an indwelling jugular vein cannula were dosed by oral gavage (po) or intravenously (iv) via the tail vein to doses corresponding to those that HTPK predicted would produce serum concentrations in the range of bioactivity observed in ToxCast bioassays. Serial blood samples were collected out to 96 h. Plasma was isolated by centrifugation and stored at -80 oC until analyzed for parent compound by HPLC-MS/MS. For po exposure, HTPK predictions for Cmax were within 3-fold of measured values for 36% of observations. HTPK over-predicted Cmax (a risk-conservative error) for 71% of observations. The prediction of Vd was within 3-fold for 43% of observations, but half-life was >3-fold over-predicted for 87% of observations. The data suggest that models based on *in vitro* HTPK data may be sufficient for ~40% of the chemicals analyzed in this study. However, for most of the discordant chemicals, the bias present for the HTPK data was conservative in a risk context. The data suggest that the discordance for many chemicals may be due to overestimation of half-life. Additional data including bioavailability and effect of transporters may aid in rendering these assays and models more predictive in the future. (This abstract does not represent US EPA policy.)

3437 High Throughput Determination of Critical Human Dosing Parameters

C. I. Nicolas[4,5], B. L. Ingle[4], M. Bacolod[1], J. Gilbert[1], B. A. Wetmore[2], C. L. Ring[4,5], R. W. Setzer[4], R. Tornero-Velez[4], M. T. Martin[4] and J. F. Wambaugh[4]. *[1]Cyprotex US, LLC, Watertown, MA; [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC; [3]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC; [4]National Exposure Research Laboratory, US EPA, Research Triangle Park, NC and [5]Oak Ridge Institute for Science and Education, ORAU, Oak Ridge, TN.*

High throughput toxicokinetics (HTTK) is a rapid approach that uses *in vitro* data to estimate TK for hundreds of environmental chemicals. Reverse dosimetry (i.e., reverse toxicokinetics or RTK) based on HTTK data converts high throughput *in vitro* toxicity screening (HTS) data into predicted human equivalent doses that can be linked with biologically relevant exposure scenarios. Thus, HTTK provides essential data for risk prioritization for thousands of chemicals that lack TK data. One critical HTTK parameter that can be measured *in vitro* is the unbound fraction of a chemical in plasma (Fub). However, for chemicals that bind strongly to plasma, Fub is below the limits of detection (LOD) for high throughput analytical chemistry, and therefore cannot be quantified. A novel method for quantifying Fub was implemented for 85 strategically selected chemicals: measurement of Fub was attempted at 10%, 30%, and 100% of physiological plasma concentrations using rapid equilibrium dialysis assays. Varying plasma concentrations instead of chemical concentrations makes high throughput analytical methodology more likely to be successful. Assays at 100% plasma concentration were unsuccessful for 34 chemicals. For 12 of these 34 chemicals, Fub could be quantified at 10% and/or 30% plasma concentrations; these results imply that the assay failure at 100% plasma concentration was caused by plasma protein binding for these chemicals. Assay failure for the remaining 22 chemicals may be due to chemical insolubility, susceptibility to enzymatic or other degradation, and ability to bind to assay plate walls. ~35% of missing Fub values were captured as a result of using this new approach and would have been missing with the use of previous HTTK protocols. This abstract does not necessarily reflect US EPA policy.

3438 Application of a Physiologically-Based Absorption Model to Characterize the Rate and Extent of Release and Absorption of Oral Extended-Release Methylphenidate

X. Yang[1], J. Duan[2], A. Jackson[2] and J. Fisher[1]. *[1]US FDA, Jefferson, AR and [2]US FDA, Silver Spring, MD.*

Our recently published physiologically based pharmacokinetic (PBPK) model for immediate-release (IR) methylphenidate (MPH) was modified to quantitatively characterize the oral absorption of extended-release (ER) MPH formulations. The mean plasma concentration-time profiles found in the literature for ER MPH following oral administration were used to calibrate the deterministic model for ER MPH. The oral systemic absorption of ER MPH formulations was described using slightly modified oral absorption code used in ADME Workbench (AEgis, Inc) (Pang S, Drug Metabolism and Disposition, 31:1507-1519, 2003). The gastrointestinal (GI) tract was divided into nine anatomically defined sections from the stomach to the small intestine and colon. The fate of ER MPH in each section is described as a function of release from the formulation, dissolution in the lumen, passive diffusion across cellular membranes, metabolism and absorption within enterocytes, and transit within the GI tract. Physiological information related to the GI tract and physicochemical properties of MPH as well as formulation related factors were gathered from the literature or predicted using *in silico* methods. To adequately fit the mean plasma time course concentrations for MPH a lag time was required and an additional rate constant to account for the release of ER MPH formulation into the GI tract lumen. The development of a deterministic ER MPH physiological absorption model will provide a quantitative tool to understand better the biological and formulation-based factors that influence the disparate pharmacokinetic plasma time course profiles reported for ER MPH.

3439 Pharmacokinetic Profiles of Perfluorobutane Sulfonate and Activation of Hepatic Genes in Mice

J. Rumpler[1], K. Das[4], C. Wood[4], M. Strynar[4], A. Lindstrom[4], J. Wambaugh[4] and C. Lau[4]. *[1]Oak Ridge Institute for Science and Education, Research Triangle Park, NC; [2]Human Exposure and Atmospheric Sciences Division/NERL, US EPA, Research Triangle Park, NC; [3]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC and [4]Toxicity Assessment Division/ NHEERL, US EPA, Research Triangle Park, NC.*

Polyfluoroalkyl substances (PFAS) are organic chemicals with wide industrial and consumer uses. They are found ubiquitously in the environment and are detectable in humans and wildlife. Perfluorobutane Sulfonate (PFBS) is a short-chained PFAS used to replace perfluorooctane sulfonate in commerce. In general, the rate of clearance for the short-chained PFAS are faster than the long-chained congeners. This study evaluated the pharmacokinetic properties of PFBS and its hepatic transcriptional responses in CD-1 mice. Males and females were given PFBS by oral gavage at 30 or 300 mg/kg; controls received water vehicle. Trunk blood, liver and kidney were collected at 0.5, 1, 2, 4, 8, 16, 24 and 48 h, and urine at 24 h. Serum, urine and tissue concentrations of PFBS were determined by HPLC-MS-MS. Expression of hepatic nuclear receptor target genes was determined by qPCR. The half-life of PFBS was estimated at 4.6 h in the males and 2.5 h in the females. The volume of distribution was similar between the two sexes (0.36-0.38 L/kg). Rate of PFBS clearance was linear with exposure doses. Within 24 h, >95% of serum PFBS was excreted into urine. PFBS was not accumulated in liver or kidney, as tissue levels of the chemical did not exceed those of serum. At 24 h after administration of 300 mg/kg dose, PFBS elevated expression of several hepatic genes targeted for PPARα and PPARγ, but not for AhR or CAR, with responses more robust in males than females. Little to no transcriptional response was seen with the low dose. The short serum half-lives of PFBS in the mouse were similar to those reported for the rat. Although PFBS did not accumulate appreciably in the liver, activation of hepatic nuclear receptors may represent one of the acute responses to the chemical at a high dose. This abstract does not necessarily reflect US EPA policy.

3440 Acute Rat Inhalation Pharmacokinetic Study to Support Occupational Exposure Limits for Proteins

J. Gould[1], B. Attalla[2], I. Carvajal[1], T. Davidson[1], J. Graham[1], J. Hillegass[1], L. Sivaraman[1], S. Julien[1], A. Kozhich[1], N. Mathias[1], B. Wang[1], C. Wang[1] and H. Haggerty[1]. [1]*Bristol-Myers Squibb Co., New Brunswick, NJ and* [2]*Charles River Laboratories Preclinical Services Montreal Inc., Senneville, QC, Canada.*

Protein-based biologics are a growing therapeutic approach in the pharmaceutical industry. As part of assessing potential workplace hazards, an Occupational Exposure Limit (OEL) is developed internally for each proprietary biologic to support safe handling. Generally, biologics are considered less hazardous than small molecule pharmaceutics following inhalation (Inh) or swallowing due to suspected lower systemic bioavailability (BA) via the lung or degradation in the gastrointestinal tract. To investigate fate after Inh, rats were exposed to either one of three proteins of varying molecular weight, interleukin-23 single-chain variable fragment (IL-23 scFv; 30.1 kD), bovine serum albumin (BSA; 67 kD), B and T lymphocyte attenuator monoclonal antibody (BTLA mAb; 144 kD), or vehicle by nose-only Inh for 1 hr. A comparable study utilizing IV administration of these proteins was also completed in order to estimate absolute Inh BA. The measured experimental exposure for IL-23 scFv, BSA, and BTLA mAb was 54, 45 or 28 mg/m^3. The systemic PK profiles for IL-23 scFv and BTLA mAb after Inh exposure included an AUC(0-5h) of 0.0563 µg•h/mL or AUC(INF) of 36.2 µg•h/mL, a Cmax of 0.016 and 0.103 µg/mL, and a Tmax of 1.5 and 49 hrs, respectively. Furthermore, the t½ was 370 hrs for BTLA mAb, and not determined for IL-23 scFv. BSA was not detected in the serum at any time point, although a worst-case BA was estimated based on the assay limit of quantitation. When compared to the IV study, the systemic BA for IL-23 scFv, BSA, and BTLA mAb following inhalation was 0.55, <0.47 and 0.73%, respectively, indicating an overall systemic BA of <1% for proteins >30 kD. These data suggest not only an intrinsic decreased systemic hazard for proteins >30 kD, but also that application of a data-derived BA adjustment factor be considered for larger proteins when calculating the OEL.

3441 Toxicokinetic and Disposition Studies of Alkylated Naphthalene AN-600 in Sprague-Dawley Rats Following Oral and Dermal Administration

L. K. Low[1], M. H. Kung[1] and C. M. Palermo[2]. [1]*ExxonMobil Biomedical Sciences, Inc., Annandale, NJ and* [2]*ExxonMobil Petroleum and Chemical, Machelen, Belgium.*

AN-600 is an alkylated naphthalene material (MW 600) used as a lubricant in industrial applications. The present study was conducted to investigate the ADME of [14C]AN-600 in SD rats following single oral and dermal administrations. Oral doses of 100 and 1000 mg/kg in corn oil were administered to male rats. About 1.3% and 0.55% of these doses were absorbed into systemic circulation in 120 h, respectively. The doses were essentially unabsorbed as they passed through the GI tract and rapidly recovered in the feces during the first 24 h, with eventual total recoveries of 95.2 to 99.4% in the feces after 120 h. Only 0.02-0.04% of the radioactive dose was detected in the tissues at 120h. HPLC-radiometric analysis and hydrolysis experiments indicated the presence of glucuronide and sulfate conjugated urinary metabolites. The biotransformation pathways most likely involve oxidative metabolism of the alkyl side chain group to the corresponding alcohol or carboxylic acid metabolites and subsequent conjugation. Feces contained exclusively unchanged parent material. The blood kinetic data were shown to best fit an oral two-compartment TK model. Dermal administration was carried out with a single dose (3.5 µl/cm2)(equivalent to 100 mg/kg b.w.) of neat [14C]AN-600. Systemic absorption was found to be only 0.16% after 120 h. Little if any radioactivity could be detected in blood or internal tissues. The dermal dose was quantitatively recovered unabsorbed at the application site. In conclusion, AN-600 was very poorly absorbed into systemic circulation following oral administration in SD rats and even less so by the dermal route. Due to its extremely low bioavailability, AN-600 is not expected to pose a systemic health risk by these routes, which has been confirmed by no systemic or reproductive toxic effects observed in 90-day dermal or two-generation reproduction (oral) toxicity studies in rats. The very poor bioavailability is explained by the physicochemical characteristics of AN-600 [i.e., high MW 600, low water solubility (<0.001 mg/L) and high log Kow (>10)] which severely limit absorption.

3442 An *In Vivo* Toxicokinetic Comparison Study Following Gavage and Dietary Administration of Alpha-Terpineol

A. Patel[2], V. T. Politano[1] and A. M. Api[2]. [1]*Coty, Inc, Morris Plains, NJ and* [2]*Research Institute for Fragrance Materials, Woodcliff Lake, NJ.* Sponsor: A. M. Api.

Previous studies on terpineol, a fragrance ingredient found in nature, indicates differences concerning the male reproductive system following gavage and dietary administration to rats. This was proposed to occur due to varying peak plasma concentrations required for adverse effects following gavage and dietary administration of terpineol. Thus a toxicokinetic comparison study was conducted to determine the underlying differences following gavage and dietary administration of terpineol. All studies were conducted according to the OECD 417 guidelines. A single dose gavage toxicokinetic study was conducted on [14C]-alpha-terpineol at doses of 75, 250 and 750 mg/kg/day to Crl:CD(SD) male rats. In another study, daily dietary non-radiolabeled terpineol (650, 2200 and 6500 ppm) was administered to male Crl:CD(SD) rats for 13 days followed by dietary [14C]-alpha-terpineol administration on day 14. The results show most of the radioactivity to be excreted in the urine and feces within 48 hours. Excretion was mainly via urine accounting for about 60% of administered gavage or dietary dose. Gavaged rat alpha-terpineol tissue: plasma concentrations remained low for all tissues with the exception of kidney and liver at all times and fat at later times. Whereas rats fed dietary alpha-terpineol had low tissue: plasma concentrations for all tissues with the exception of kidney, liver and fat at all times. The whole blood: plasma concentrations suggested relatively little distribution into red blood cells. Unchanged alpha-terpineol represented a maximum of 18.7% dose in the feces and 0.1% dose in urine for the gavage route and a maximum of 12.4% dose in feces and <0.1% in the urine for the dietary route. The four major metabolites identified were glucuronide conjugates of alpha-terpineol or glucuronide conjugates of hydroxyl alpha-terpineol. The results concluded that the peak plasma concentrations remained 9-9.8 times lower for rats on dietary treatment as compared to oral gavage.

3443 Oral and Intravenous Pharmacokinetics of NVN1000, a Nitric Oxide Donating Macromolecule in Miniature Swine

K. Coggan, A. Weston, A. Radwan, J. Goldbaum, B. Privett, C. Geer, N. Stasko and S. Hollenbach. *Novan Therapeutics, Durham, NC.* Sponsor: S. Anderson.

NVN1000 is a nitric oxide (NO) releasing polysiloxane macromolecule that has demonstrated both antimicrobial and anti-inflammatory effects *in vitro* and *in vivo* and is currently in clinical development for the treatment of acne vulgaris. NVN1000 contains the NO donating moiety NMethylaminopropyl-trimethoxysilane-diazeniumdiolate (MAP3-NONOate) and release of NO from this macromolecule is proton initiated. In the blood, endogenous and exogenous NO rapidly reacts with hemoglobin to produce methemoglobin (metHb) and is also metabolized to form nitrate. Hydrolyzed MAP3 (hMAP3) is a unique and quantifiable analyte of NVN1000 exposure. Additional surrogate analytes include increases in elemental silicon, plasma nitrate and methemoglobin. The objective of this study was to determine a quantitative correlation between these analytes after oral (PO) and intravenous (IV) administration in Hanford minipigs. Animals received a single administration of NVN1000 at 20 mg/kg (IV), 30 or 100 mg/kg (PO). Serial blood samples were collected via jugular vein catheters predose and up to 48 hours postdose. For all four analytes, the IV t1/2 was ≤ 4.5 hrs. The linear association (r2) between hMAP3 and silicon concentrations was 0.99 where a 0.42 ng/mL increase in silicon correlated to a 1 ng/mL increase in hMAP3; r2 for hMAP3 and nitrate association was 0.91 where a 0.31 ng/mL increase in nitrate correlated to a 1 ng/mL increase in hMAP3. There was a linear association (r2 > 0.8) between nitrate and metHb concentrations at 20 mg/kg IV, 100 mg/kg PO, and when all three dose levels were combined. In this model, a systemic nitrate increase >1400 ng/mL would be required for an absolute 1% increase in metHb. These correlations show tight associations between the active and inactive components of the NVN1000 molecule as it is metabolized *in vivo*. As hMAP3 is not endogenous, small changes in hMAP3 concentrations can be quantitated, making it the more appropriate analyte to demonstrate NVN1000 systemic exposure.

3444 Disposition of Ethylene Glycol 2-Ethylhexylether Following Oral Administration and Dermal Application to Harlan Sprague Dawley Rats and B6C3F1/N Mice

B. C. Moeller[1], M. Doyle-Eisele[1], J. D. McDonald[1], C. R. Blystone[2] and S. Waidyanatha[2]. *[1]Lovelace Respiratory Research Institute, Albuquerque, NM and [2]Division of the National Toxicology Program, NIEHS, Research Triangle Park, NC.*

Ethylene glycol 2-ethylhexylether (EGEHE) is a solvent used in a variety of products including paints, sealants and cleaners. In support of toxicity testing of EGEHE, disposition and metabolism studies were conducted in Harlan Sprague Dawley rats and B6C3F1/N mice following a single oral (50, 150 or 500 mg/kg) or dermal exposure (50 mg/kg) to [14C]EGEHE. In male rats, majority of the oral dose was recovered in urine (54-73%), feces (14-17%) and as CO2 (2-7%) within 48 or 72h. There was no apparent dose-related effect in the disposition of EGEHE in male rats. Absence of radioactivity in 24h bile suggested that observed fecal radioactivity was due to the unabsorbed dose. The disposition was similar in female rats (urine, 68%; feces, 7%) following oral exposure to 50 mg/kg. In male and female mice, following oral exposure to 50 mg/kg, the administered radioactivity was recovered in urine (27-48%), feces (17-32%), and as CO2 (4.6-5.8%); however, unlike in rats, excretion via exhaled volatile organic chemicals (VOCs) was significant in mice (20%). Following dermal application to a covered dose site in male and female rats (35-37%) and mice (22-24%) absorption was moderate at 72 h; excretion was mainly via urine (rats, 29-31%; mice, 12-14%), and feces (rats, 3%; mice, 4-8%). A significant percent of the administered dose was recovered as exhaled VOCs (32-58%) in both rats and mice, likely due to evaporation from the dose site. The residual radioactivity in tissues following oral (≤ 6%) and dermal (≤ 2%) exposure was low with the highest radioactivity found in the liver (tissue: blood ratio 2-8). Profiling of urine showed that EGEHE was extensively metabolized in both species and sexes. These data suggests that EGEHE was absorbed following oral or dermal exposure, extensively metabolized, and excreted mainly via urine with some species differences in the disposition.

3445 Tetrabromobisphenol A (TBBPA); Dose- and Time-Dependent Changes in Plasma TBBPA and Its Conjugates Over 28 Days of Administration

S. Borghoff[2], D. Wikoff[1], J. E. Rager[1] and L. C. Haws[1]. *[1]ToxStrategies, Austin, TX and [2]ToxStrategies, Cary, NC.*

TBBPA, a nongenotoxic flame retardant, causes uterine tumors in female rats. A proposed mode of action (MoA) for these tumors involves disruption of estrogen-signaling pathways due to increased bioavailability of estradiol. This is thought to occur though the molecular initiating event (MIE) of binding and inhibition of SULT1E1, the sulfotransferase responsible for inactivating and enhancing the elimination of estradiol. The MIE is proposed to occur under high doses conditions. The objective of this study was to evaluate the effect of dose on the level of TBBPA, TBBPA-glucuronide (GA) and TBBPA-sulfate (S) conjugates in female Wistar Han rat plasma following 28-days of exposure to TBBPA (50, 100, 250, 500 or 1000 mg/kg), thus characterizing conjugation at high doses (such as those used in the cancer bioassay). Blood samples were collected at 4- and 8- hrs post-dosing on study day 7, 14 and 28; plasma was analyzed for TBBPA, TBBPA-GA and TBBPA-S by LC/MS-MS. There was an increase in TBBPA, TBBPA-GA, and TBBPA-S plasma concentrations at 4-hrs post dose on day 7, 14 and 28; by 8-hrs post dose, plasma concentrations of all three analytes were decreased compared to the 4-hr time point. However, there was still an increase in plasma concentration of TBBPA-GA and TBBPA-S at 28 days compared to concentrations on day 7 and 14 at doses ≥ 250 mg/kg suggesting induction of glucuronidation and sulfation. At all TBBPA doses and time points, there was a lower plasma concentration of TBBPA-S compared to TBBPA-GA; with the S/GA ratio decreasing with dose. This effect was most apparent at 28-days of administration. Together these data show that administration of the high doses of TBBPA associated with the induction of uterine tumors results in a decrease in the sulfation of TBBPA compared to glucuronidation. A decrease in TBBPA sulfation pathway supports *in vitro* data of TBBPA inhibition of SULT1E1 activity. Furthermore, findings suggest that the proposed MoA involving increased estradiol bioavailability via inhibition of estradiol sulfation by TBBPA is plausible.

3446 Intravenous and Oral Toxicokinetics of Domoic Acid in Non-Human Primates

R. L. Petroff[1], J. Jing[1], B. Crouthamel[2], K. Grant[1], N. Isoherranen[1] and T. Burbacher[1]. *[1]Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and [2]Infant Primate Research Laboratory, Washington National Primate Research Center, Seattle, WA; [3]Pharmaceutics, University of Washington, Seattle, WA.* Sponsor: T. Burbacher.

Domoic acid (DA), a potent neurotoxin produced by algae from the Pseudo-nitzschia family, presents an emerging threat to the health of shellfish consumers. Though heavily studied in rodent models, the disposition characteristics of DA in human ingestion of amounts near the EPA's TDI, 0.075 mg/kg, are principally unknown. Thus, as part of a larger neuronal-fetal study, we worked to determine the bioavailability and distribution kinetics of an acute low dose in a species closely related to humans, to potentially model and predict human dose-exposure relationships. Three healthy, adult, female *Macaca fascicularis* were intravenously injected with 0.005 mg/kg of DA and given oral doses of 0.05, 0.15, and 0.45 mg/kg of DA. Females were dosed either with a delayed feeding schedule, 90 minutes post-delivery, or with food. Blood plasma was collected at eleven time points, up to 24 hours, and DA concentrations were measured via LC-MSMS. The IV study produced data consistent with findings from previous work, with no visible observed side effects. The disposition of DA displayed two-compartmental kinetics with a terminal half-life of one hour and levels below detection limit at four hours. Additionally, the clearance of DA matched the glomerular filtration rate in macaques. Following oral administration, a prolonged exposure was observed with a terminal half-life upwards of 18 hours. The bioavailability of DA was 12-15%. Interestingly, when DA was administered with food, bioavailability, absorption, and absorption rate dramatically decreased, indicating a pronounced food effect on DA absorption. The first of its kind, this study not only demonstrated a twofold higher oral bioavailability in primates than previously hypothesized, but also revealed data that supports a strong food effect with DA administration. This research was funded by NIH grants P51OD010425 and 1R01ES023043-01A1.

3447 Determination of Concentration and Free Fraction Dependent Uptake of Deltamethrin (DLM) in Adult Male Sprague-Dawley Rats Using an *In Situ* Brain Perfusion Technique

M. Amaraneni[4], J. Pang[4], T. G. Osimitz[2], D. Gammon[1], M. R. Creek[3], C. A. White[4], B. S. Cummings[4] and J. V. Bruckner[4]. *[1]FMC Corporation, Ewing, NJ; [2]Science Strategies, Charlottesville, VA; [3]Valent USA. Corporation, Dublin, CA and [4]Pharmaceutical and Bio-medical Sciences, University of Georgia at Athens, Athens, GA.*

It is important to assess the extent to which a chemical in the systemic circulation gains access to the brain, especially for potentially neurotoxic compounds such as pyrethroids. We evaluated the uptake of DLM into the brain as a function of its free fraction, as well as its concentration. 14C-DLM in HBSS buffer with different human serum albumin (HSA) concentrations (2-5%) was infused through the left common carotid artery @ 500 µl/min for 2 min, using a Harvard infusion pump. The left half of the brain was then collected, processed and analyzed for DLM by liquid scintillation counting. Infusion of 1µM DLM in 2% HSA resulted in left brain levels of 13.8 pmol/g, whereas levels of 11, 12.8 and 10.6 pmol/g were measured following infusion in 2.9, 4 and 5% HSA, respectively. Thus, brain uptake of DLM did not vary in this physiological range of albumin concentrations. We have previously found that the free fraction of DLM is consistently 10% in this range. Infusion of 1, 10 and 50 µM 14C-DLM with 4% HSA resulted in left brain levels of 12.8, 137.0 and 574.1 pmol/g respectively. Prior infusion of mannitol increased the uptake of 50µM 14C-DLM from 574.1 to 1754.4 pmol/g, whereas the uptake of 1µM 14C-DLM was not significantly altered. Thus the role of BBB in opposing the entry of DLM into the brain is only limited to very high concentrations of DLM where the binding is saturated. In summary, these data show that brain uptake of environmental concentrations of DLM is not influenced by physiological changes in HSA concentration. The uptake of DLM is dependent on its concentration and is linear in the range tested. Supported by the Council for the Advancement of Pyrethroid Human Risk Assessment.

3448 Sex-related Toxicokinetic (TK) Study of CIS-Permethrin in Adult and Weanling Sprague-Dawley (SD) Rats

J. Pang[3], T. B. Mortuza[3], S. Muralidhara[3], M. Amaraneni[3], M. R. Creek[4], D. Gammon[2], S. S. Anand[1], B. S. Cummings[3], J. V. Bruckner[3] and C. A. White[3]. *[1]Dupont Haskell, Newark, DE; [2]FMC Corporation, Ewing, NJ; [3]University of Georgia, Athens, GA and [4]Valent USA., Dublin, CA.*

Permethrin, a mixture of its cis (CIS) and trans (TRANS) isomers, is a type I pyrethroid. Despite its extensive use in agriculture and households for pest control in the US, research has seldom been conducted to assess potential gender differences in its toxicokinetics. The current experiments were carried out to investigate whether significant differences exist between male and female SD rats in blood and tissue levels of CIS following oral dosing. Adult and weanling (21-day-old) SD rats of both sexes were administered a single oral bolus of 60 mg CIS/kg in 5 mL/kg of corn oil. Serial sacrifices were performed (N=4/time-point for adult males, N=3/time-point for adult females, and N=4/time-point for mixed-gender pups) 1, 2, 4, 6, 8, 12, and 24 hr post dosing. Plasma, brain, liver, muscle, and fat samples were collected for analysis by HPLC. CIS concentrations in liver were modestly higher in female rats in both the adults and 21-day-old pups. Liver area under the curve values were 12.7 and 40.3 h•µg/ml for adult male and female rats, respectively. However, the CIS levels in plasma, brain and muscle did not show any difference between female and male rats in either weanlings or adults. Furthermore, no significant gender-related difference was observed in fat CIS levels in the adults. Weanlings had no visible body fat. In conclusion, gender-related differences were observed only in liver, which may be related to the metabolic differences between male and female rats. P-450 catalyzed metabolism is generally significantly higher in adult male rats, and CIS is both oxidized by P450s and hydrolyzed by carboxylesterases. Supported by the Council for Advancement of Pyrethroid Human Risk Assessment (CAPHRA).

3449 Thifensulfuron-Methyl Dosimetry Assessment for Female Sprague-Dawley Rats During Gestation

M. W. Himmelstein[2], L. A. Malley[1] and S. R. Frame[2]. *[1]DuPont Crop Protection, Newark, DE and [2]DuPont Haskell, Newark, DE.*

Thifensulfuron-methyl (TSM) was previously tested for developmental (DT) and reproductive (RT) toxicity, and PK disposition of 14C residues in rats. The objective was to use existing data from the PK, DT and RT studies (each with different exposure paradigms) to support the absence of a compound-related effect on offspring kidney development in the DT study. In the two-generation RT study dams were continuously administered treated diet throughout gestation and lactation; parameters evaluated included gross and microscopic examination of the kidneys of male and female weanling rats from the F2b generation. No gross or microscopic changes were observed in the kidney of the weanling rats at maternal dietary concentrations up to 2500 ppm, (221 mg/kg/day). In the DT study, dams were administered gavage doses up to 800 mg/kg/day during GD 6-15. On GD21, the incidence of fetuses with small renal papilla was slightly increased at this dose. In the PK studies 14C-TSM was administered at 20 or 2000 mg/kg. Urine was a primary excretory route, and un-metabolized TSM was the major identified component. Elimination was dose proportional, first-order kinetics. The predicted blood t1/2 = 12-19 hr. Measured blood concentrations at 96 hr after dosing and the t1/2 prediction were used to back-calculate blood TSM concentrations at relevant times for the toxicity studies. By GD19 in the DT study (i.e., 96 hr following the last day of dosing on GD15), blood concentrations at the 800 mg/kg/day dose are predicted to have declined to 0.5 µg/g. In the RT study, where dosing was continuous at a dietary intake of 221 mg/kg/day, predicted blood concentrations are maintained at 4.4 µg/g. These results support the conclusion that the observation of small or absent renal papilla in the DT study with TSM were not test substance-related but were spurious findings resulting from the high variability in renal papillary development in late gestation rat fetuses.

3450 Development of HepG2-Derived Cells Expressing Cytochrome P450s for Assessing Metabolism-Associated Drug-Induced Liver Toxicity

J. Xuan[2], S. Chen[2], B. Ning[2], W. H. Tolleson[2] and L. Guo[2]. *[1]Division of Biochemical Toxicology, National Center for Toxicological Research, US FDA, Jefferson, AR and [2]Division of System Biology, National Center for Toxicological Research, US FDA, Jefferson, AR.*

The generation of reactive metabolites from therapeutic agents is one of the major mechanisms of drug-induced liver injury (DILI). In order to evaluate metabolism-related toxicity and improve drug efficacy and safety, we generated a battery of HepG2-derived cell lines that express 14 cytochrome P450s (CYPs) (1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5 and 3A7) individually using a lentiviral expression system. The expression/production of a specific CYP in each cell line was confirmed by an increased abundance of the CYP at both mRNA and protein levels. Moreover, the enzymatic activities of representative CYPs in the corresponding cell lines were also measured. Using our CYP-expressed HepG2 cells, the toxicity of three drugs that could induce DILI (amiodarone, chlorpromazine and primaquine) was assessed, and all of them showed altered (increased or decreased) toxicity compared to the toxicity in drug-treated wild-type HepG2 cells. CYP-mediated drug toxicity examined in our cell system is consistent with previous reports, demonstrating the potential of these cells for assessing metabolism-related drug toxicity. This cell system provides a practical *in vitro* approach for drug metabolism screening and for early detection of drug toxicity. It is also a surrogate enzyme source for the enzymatic characterization of a particular CYP that contributes to drug-induced liver toxicity.

3451 NADPH-Cytochrome P450 Reductase-Mediated Denitration Reaction of 2,4,6-Trinitrotoluene to Yield Nitrite in Mammals

Y. Shinkai[2], T. Kikuchi[2] and Y. Kumagai[2]. *[1]Faculty of Medicine, University of Tsukuba, Tsukuba, Japan and [2]Graduate School of Environmental Sciences, University of Tsukuba, Tsukuba, Japan.*

While the biodegradation of 2,4,6-trinitrotoluene (TNT) via the release of nitrite is well established, mechanistic details of the reaction in mammals are unknown. To address this issue, we attempted to identify the enzyme from rat liver responsible for the production of nitrite from TNT. A NADPH-cytochrome P450 reductase (P450R) was isolated and identified from rat liver microsomes as the enzyme responsible for not only the release of nitrite from TNT but also formation of superoxide and 4-hydroxyamino-2,6-dinitrotoluene (4-HADNT) under aerobic conditions. In this context, reactive oxygen species generated during P450R-catalyzed TNT reduction were found to be, at least in part, a mediator for the production of 4-HADNT from TNT via formation of 4-nitroso-2,6-dinitrotoluene. P450R did not catalyze the formation of the hydride-Meisenheimer complex (H--TNT) that is thought to be an intermediate for nitrite release from TNT. Furthermore, in a time-course experiment, 4-HADNT formation reached a plateau level and then declined during the reaction between TNT and P450R with NADPH, while the release of nitrite was subjected to a lag period. Notably, the produced 4-HADNT can react with the parent compound TNT to produce nitrite and dimerized products via formation of a Janovsky complex. Our results demonstrate for the first time that P450R-mediated release of nitrite from TNT results from the process of chemical interaction of TNT and its 4-electron reduction metabolite 4-HADNT. Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (#25220103 to YK).

3452 Activation of Immune Modulators, a Novel Mechanism in Pharmacokinetics Drug-Drug Interaction Between Taxol and Irinotecan

P. Mallick Bandi and R. Ghose. *Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX.*

Inflammation-mediated impairment of drug metabolism has significant implications on disposition of clinical drugs. We have previously shown inflammation-mediated activation of Toll-like receptors 4 (TLR4) downregulates expression and activity of drug metabolizing enzymes (DMEs). Taxol is a widely used anticancer drug that can activate TLR4. In a Phase-1 study, pharmacokinetic DDI by taxol on irinotecan caused increased irinotecan-mediated diarrhea. Irinotecan is a potent anticancer drug and is converted to SN-38 by carboxylesterases (CE) which is then conjugated by Ugt1a1 to SN-38 glucuronide (SN-38G). In intestinal lumen, SN-38G is deconjugated by beta-glucuronidases to SN-38 that causes diarrhea. Therefore, we hypothesize that taxol will alter the pharmacokinetics of irinotecan and SN-38 by regulating expression of DMEs

by TLR4-dependent mechanism. Primary hepatocytes from TLR4 WT & mutant were treated with irinotecan or SN38 in presence and absence of taxol. Gene expressions of DMEs/transporters involved in irinotecan's disposition were evaluated by RTPCR and levels of SN-38 and SN-38G were analyzed in LC-MS/MS. Taxol significantly induced gene expression of IL-1β, Il-6 and TNF-α in TLR4 WT but not in mutant, establishing TLR-4 activation. The gene expression of Cyp3a11 (~600 folds) and Ugt1a1 (~4 folds) were induced by taxol via TLR4-dependent mechanism, however CE activity remained unaltered. Gene expression of transporters; Mrp2 & Mdr1b and nuclear receptor; PXR were also induced but irrespective of TLR4 activation. TLR4 dependent alterations of DMEs by taxol resulted ~2 fold higher levels of SN-38 & SN-38G when treated in combination with irinotecan and SN-38. Thus, our result determines TLR4 as a novel mechanism in DDI between taxol and irinotecan and warrants dose adjustments for patients with TLR4 polymorphism.

3453 Metabolism and Disposition of Sulfolane in Male and Female Harlan Sprague Dawley Rats and B6C3F1/N Mice

T. R. Fennell[2], S. Black[2], S. Watson[2], P. Patel[2], R. Snyder[2], J. Burgess[2], C. R. Blystone[1] and S. Waidyanatha[1]. *[1]2Division of the National Toxicology Program, Research Triangle Park, NC and [2]RTI International, Research Triangle Park, NC.*

Sulfolane is used in oil and gas refining, and has been found as a ground water contaminant in Alaska. In support of the toxicity testing of sulfolane by the National Toxicology Program, metabolism and disposition studies were conducted following gavage administration (30, 100 or 300 mg/kg) and dermal application (100 mg/kg) of [2,5-14C]sulfolane to male and female Harlan Sprague Dawley Rats and B6C3F1/N Mice. Urine, feces, exhaled CO2 and volatiles were collected for up to 72 h after dosing. In rats, 86-95% of the administered radioactivity was recovered in urine following gavage administration, and 1% each of the dose was recovered in feces and in tissues; radioactivity recovered as CO2 and exhaled volatiles was negligible. In mice following gavage administration (100 mg/kg), approximately 86 and 63% of the administered radioactivity was found in urine and 8 and 30% in feces, in males and females, respectively. Following dermal application of 100 mg/kg to a covered dose site in male and female rats, 13-16% of the dose was recovered in urine, 1% in feces, and 2% in tissues; the recovery of radioactivity from the dose site was 2-5%. The majority of the applied dose was recovered from the dermal appliance (47-59%). In contrast in mice, 62-69% of a dermal dose was recovered in urine and 7-9% in feces suggesting higher dermal absorption than in rats. Profiling of urine showed that a single metabolite accounted for the majority of the radioactivity in urine in rats and mice (55-97%), and the remainder was primarily from unchanged sulfolane (0-40%). The metabolite was identified as 3-hydroxysulfolane using a combination of mass spectrometry and NMR spectroscopy. This study indicated similar disposition and metabolism *in vivo* in male and female rats and mice, with no apparent sex difference, but with greater dermal uptake in mice.

3454 Free Radical Metabolite of Munition RDX (Hexahydro-1,3,5-Trinitro-1,3,5-Triazine) and 1-Nitroso Degradation Product MNX Determined with Spin Trapping and Electron Paramegnetic Resonance (EPR)

R. J. Al Suliman[3], K. A. El Sayed[3], A. L. Russell[1], A. J. Bednar[1] and S. A. Meyer[3]. *[1]US Army Engineer Research and Development, Vicksburg, MS; [2]Basic Pharm. Sci., University of Louisiana-Monroe, Monroe, LA and [3]Toxicology, University of Louisiana-Monroe, Monroe, LA.*

RDX and its N-nitroso environmental degradation product MNX (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine) are ground water and soil contaminants of retired DoD munitions plants. Previously, our laboratory demonstrated that RDX and MNX cause delayed-onset anemia and granulocytopenia due to loss of bone marrow progenitor cells after a single oral dose (94 mg/kg) in rats (Jaligama et al. 2013 Toxicol. Appl. Pharmacol. 266:443-51). Myelosuppression of N-nitroso bioreductive prodrugs, e.g., prototype tirapazamine, is thought due to reductive metabolism to free radical in hypoxic bone marrow niches. In this study, we hypothesize that RDX and MNX undergo reductive metabolism to free radical(s), potential mediators of the observed hematotoxicity. Methods: RDX and MNX (50μM) were incubated with female Sprague-Dawley rat liver microsomes (0.25 mg/ml in 100 mM phosphate buffer and 5mM magnesium chloride, pH 7.4) and NADPH (0.5 mM) under aerobic and anaerobic conditions (N2 bubbling for 15 min). Samples were quenched at 0-60 min and run on RP (C18)-HPLC to determine the loss of RDX and MNX. Electron paramagnetic resonance (EPR) spectroscopy with spin trap 4-pyridyl-1-oxide-N-tert-butylnitrone (4-POBN, 10mM, Tris-HCl 10mM pH 7.4) was used to detect free radicals. Incubation under anaerobic conditions led to metabolism with rates of loss of RDX ~4X greater than for MNX. Free radical(s) were detected and levels were comparable for RDX and MNX. Metabolism of RDX and MNX to more polar product(s) and free radical(s) was NADPH-dependent. Negligible RDX and MNX loss and free radical production occurred with aerobic conditions. We conclude that both RDX and MNX are reduced by microsomal NADPH dependent reductase(s) to free radicals that have potential to mediate observed hematotoxicity.

3455 Human Sulfotransferases Enhance the Cytotoxicity of Tolvaptan

J.-L. Fang, Y. Wu, G. Gamboa da Costa, S. Chen, P. Chitranshi and F. A. Beland. *NCTR/US FDA, Jefferson, AR.*

Tolvaptan, a vasopressin receptor 2 antagonist used to treat hyponatremia, has recently been reported to be associated with a risk of liver injury. Sulfotransferases (SULTs) have been implicated as important detoxifying and/or activating enzymes for numerous xenobiotics, drugs, and endogenous compounds. To characterize better the role of SULTs in tolvaptan metabolism, HEK293 cells stably overexpressing 12 human SULTs were generated. Using these cell lines, the extent of tolvaptan sulfate formation was assessed by reversed-phase high-performance liquid chromatography through comparison to a synthetic standard. Of the 12 known human SULTs, no detectable sulfation of tolvaptan was observed with SULT1A1, SULT1A2, SULT1A3, SULT1C2, SULT1C4, SULT4A1, or SULT6B1. The affinity of individual SULT isozymes, as determined by Km analysis, was SULT1C3 >> SULT2A1 > SULT2B1 ~ SULT1B1 > SULT1E1. The half inhibitory concentration of tolvaptan on cell growth in HEK293/SULT1C3 cells was significantly lower than that in HEK293/vector cells or HEK293 cells. Moreover, exposing cells to tolvaptan in the presence of cyclosporine A, an inhibitor of the drug efflux transporters, significantly increased the intracellular levels of tolvaptan sulfate and decreased the cell viability in HEK293/SULT1C3 cells. These data indicate that sulfation is associated with the toxicity of tolvaptan.

3456 Protein-Protein Interaction of Cytochrome P450 (CYP) 3A4 and UDP-Glucuronosyltransferase (UGT) 1A7: The Involvement of 208th Residue of UGT1A7 in the Functional Interaction and Evidence for the Physical Interaction in Living Cells by Fluorescent Resonance Energy Transfer (FRET) Analysis

N. Egoshi[5], K. Kinoshita[5], H. Koba[5], T. Takeda[5], S.-i. Ikushiro[4], K. Nagata[2], Y. Yamazoe[1], P. I. Mackenzie[3], H. Yamada[5] and Y. Ishii[5]. *[1]The Cabinet Office, Government of Japan, Tokyo, Japan; [2]Tohoku Pharmaceutical University, Sendai, Japan; [3]Department of Clinical Pharmacology, Flinders Medical Centre, Adelaide, Australia; [4]Faculty of Engineering, Toyama Prefectural University, Toyama, Japan and [5]Graduate School of Pharmaceutical Science, Kyushu University, Fukuoka, Japan.*

Purpose: We have recently shown that CYP3A4 alters the function of some allelic variants of UGT1A7: for instance, CYP3A4 enhances the Vmax of UGT1A7*1 (wild-type) and *2 (N129K, R131K), whereas the reverse effect was seen for UGT1A7*3 (N129K, R131K, W208R). In this study, we examined 1) the effect of W208R mutation in UGT1A7*4 in the functional interaction with CYP3A4 and 2) the protein-protein interaction of UGT1A7 and CYP3A4 in living cells by using FRET. Method: A baculovirus-Sf9 system was used to establish single and dual expression of UGT1A7*1/*4 (W208R) and CYP3A4. The level of UGT expression in microsomes was determined by western blotting, and the comparable content of UGT1A7 protein among preparations was used in the assay of glucuronidation activity toward SN-38, an active metabolite of irinotecan. For FRET analysis, fluorescent proteins, AcGFP and mCherry, were fused to UGT1A7*1/*4 and CYP3A4, respectively. The constructs for UGT-AcGFP and CYP3A4-mCherry were co-transfected in COS cells. Result: Although the functions of wild-type UGT1A7*1 and an allelic variant *4 were comparable, they exhibited the reverse effect on the Vmax of CYP3A4: for instance, the Vmax values of UGT1A7*1 and *4 were enhanced and reduced, respectively, by CYP3A4. In accordance with these functional interactions, FRET was observed between UGT1A7*1/*4-AcGFP and CYP3A4-mCherry. Conclusion: The 208th residue in UGT1A7 plays a determining role whether CYP3A4 alters this UGT positively or negatively. It is also demonstrated that not only wild-type UGT1A7*1 but also the allelic variant *4 associate with CYP3A4 in living cells.

3457 Cytosolic Sulfotransferase Expression and Regulation in Cell Culture Models of Human Liver Development

K. G. Barrett[2], Z. Duniec-Dmuchowski[2], A. Soto-Gutiérrez[1], T. Kocarek[2] and M. Runge-Morris[2]. *[1]Department of Pathology, University of Pittsburgh, Pittsburgh, PA and [2]Institute of Environmental Health Sciences, Wayne State University, Detroit, MI.*

Sulfonation is a major biotransformation process in the developing fetus, but there is limited information about the expression and regulation of the cytosolic sulfotransferases (SULTs) during this life window. Using HepaRG cells as a model of human liver development, we characterized the temporal patterns of SULT expression as the bipotent cells were cultured through a proliferative stage, a confluent but relatively undifferentiated stage, and a more differentiated hepatocyte-like stage. Four patterns of SULT expression, as assessed by mRNA content, were observed. SULT1A1 expression was relatively constant throughout the differentiation process, while SULT2A1 expression increased with differentiation. SULT1B1, 1C2, 1C3, 1C4, and 1E1 expression increased from proliferation to confluency, but decreased during differentiation. SULT2B1 expression was slightly higher during the proliferative stage than during differentiation. We also characterized SULT regulation by nuclear receptors in primary cultured human fetal hepatocytes. The most abundant SULT mRNAs in the fetal hepatocytes were SULT1C2, 1C4, 1E1, and 2A1, while SULT1A1, 1B1, and 2B1 mRNAs were present at lower levels. Treatment with GW3965 (liver X receptor agonist) significantly increased SULT1A1 expression; rifampicin (pregnane X receptor), GW4064 (farnesoid X receptor), and rosiglitazone [peroxisome proliferator-activated receptor (PPAR) g] increased SULT1B1; rosiglitazone and 1α,25-dihydroxyvitamin D3 (vitamin D receptor) increased SULT1C2; and GW7647 (PPARα) increased SULT2A1. These findings demonstrated that the temporal expression of SULTs during HepaRG cell differentiation resembles some of the SULT expression patterns that occur during human liver development, and suggest that nuclear receptors likely regulate SULT expression during fetal life. This work was supported by NIEHS grant R01ES022606 and Center Grant P30ES020957.

3458 Impact of Hepatic P450-Mediated Biotransformation on Airway Epithelial Repair Following Naphthalene Inhalation

N. Kovalchuk[3], L. Van Winkle[2], Q.-Y. Zhang[3] and X. Ding[1]. *[1]College of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY; [2]UC Davis, Davis, CA and [3]Wadsworth Center, New York State Department of Health and School of Public Health, State University of New York, Albany, NY.*

Naphthalene (NA) is a ubiquitous air pollutant classified as a Possible Human Carcinogen with non-genotoxic potential. Prerequisite for NA toxic effects in nasal and airway epithelia is metabolic bioactivation by P450 enzymes. While NA is bioactivated in both the lung and the liver, and its metabolites circulate throughout the body, only the conducting airway epithelium of the lung exhibits naphthalene necrosis in mice. The objective of this study was to evaluate the effects of a compromised hepatic metabolism of inhaled NA on airway repair. We hypothesized that the loss of hepatic P450 activity will lead to increased amounts of NA available for site-specific metabolism by lung P450s, which will intensify epithelial damage and change the extent and timing of reparative cell proliferation in the airways. To test this hypothesis, we compared the BrdU and Ki67 labeling indices (LI) in the airway regions of wild-type (WT) mice to those of liver-Cpr-null (LCN) mice at different post-exposure time following NA inhalation at an occupationally relevant dose (10 ppm). We found that epithelial cell BrdU incorporation was significantly lower in distal (but not proximal) airways of LCN mice than in WT mice at 2 days after NA exposure. However, the fraction of regenerating cells (Ki67 LI) was increased in distal airways of LCN mice, compared to WT mice, at 3 and 5 days following exposure. These data suggest that the extent of NA-induced epithelial damage in the distal airways, as reflected by the cellular proliferative response, is influenced by hepatic P450 activity. (Supported by NIEHS grant E020867)

3459 *In Vitro* Study of Toxic Effects of BDE-99 in a Zebrafish Cell Line, ZFL

J. Yang and K.-M. Chan. *School of Life Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong.*

Polybrominated diphenyl ethers (PBDEs) belong to an important class of flame-retardants used in the consumer industry and often found to be associated with electronic waste disposals. Though tetra- and penta-BDEs have been banned for use, levels of many BDEs are increasing in wildlife and humans due to their bioaccumulation property. In Hong Kong and China, BDE-99 was predominantly found from samples in fish from the wet market and breast milk. In the present study, we used a zebrafish liver cell line (ZFL) for acute exposure test of BDE-99 to investigate the toxicities of PBDEs in the aquatic environment. Initial effort was employing real time PCR assay to profile genes encoding thyroid hormone (TH) hepatic metabolizing enzymes upon 10%, 25%, 50% of 96 h LC50 treatment (96 h LC50: 1.2 μM). It was found that cyp1a was significantly up-regulated to 44.4-fold at 24 h (short term) and 11.9-fold at 96 h (long term). To elucidate our hypothesis that BDE-99 is metabolized through aryl hydrocarbon receptor (AhR) mediated Cyp1a induction, we performed ethoxyresorufin-o-deethylase (EROD) assay at Cyp1a enzymatic level, electrophoretic mobility shift (EMSA) assay at direct AhR-XRE binding level, and AhR dependent xenobiotic response element (XRE) luciferase reporter gene assay at Cyp1a transcriptional level. In the EROD assay, BDE-99 individual exposure exhibited Cyp1a agonistic induction activity, but. And antagonistic effect was observed upon co-exposure with the well known Cyp1a inducer, benzo-a-pyrene (BaP). In EMSA assay, it was found that BDE-99 markedly enhanced the recruitment of AhR to its binding site in cyp1a promoters at 0.29 μM, and XRE5 was the predominant target. Moreover, slight but significant induction of XRE linked luciferase reporter gene activity was observed. in the developed reporter gene assay. We hence conclude that BDE-99 could induce Cyp1a activity through direct interference with AhR-XRE binding.

3460 Pro-Drug Activation and Detoxification in a Dynamic Microfluidic System

J. Kim[4], D. A. Fluri[2], R. Marchan[1], K. Boonen[3], S. Hammad[1], S. Messner[2], B. Landuyt[3], J. G. Hengstler[1], J. M. Kelm[2], A. Hierlemann[4] and O. Frey[4]. *[1]IFADO Dortmund, Dortmund, Germany; [2]InSphero AG, Schlieren, Switzerland; [3]KU Leuven, Leuven, Belgium and [4]Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland.*

Rational development of more physiological *in vitro* models includes the design of robust and flexible multi-tissue devices, which allow for studying tissue-tissue interactions. The newly developed microfluidic device developed within this consortium consists of interconnected chambers, which are loaded with pre-formed spherical microtissues. Gravity-driven flow is generated through automated chip-tilting without any need for tubing and external pumps. Two case studies will be presented: Pro-drug activation: Rat liver and human colorectal tumor microtissues were interconnected on the chip and cultured over 8 days in the presence of the pro-drug cyclophosphamide. Cyclophosphamide is cytostatic but only after bio-activation by the liver. This bio-activation-mediated effect was only observed in perfused and interconnected co-cultures of the different microtissue types on chip, whereas the discontinuous transfer of supernatant via pipetting from liver microtissues that have been treated with cyclophosphamide in culture wells did not significantly affect tumor growth. Detoxification: Human liver and iPS-derived cardiac microtissues were interconnected and cultured under continuous perfusion in the presence of the pro-drug Terfenadine. Terfenadine is bio-activated by the liver and transformed to fexofenadine, an antihistamine drug used in the treatment of allergy symptoms. Terfenadine itself is cardiotoxic at higher doses, whereas no cardiotoxicity is associated with fexofenadine. Cessation of spontaneous contraction was observed in static as well as perfusion conditions upon culturing cardiac microtissues alone. However, liver-related detoxification of Terfenadine was observed under perfusion conditions when combining liver and cardiac microtissues. In contrast, discrete transfer of supernatants from liver MTs treated with Terfenadine under static conditions did not lead to any reduction of toxic effects on the cardiac microtissues. The results indicate the utility and multi-tissue functionality of this platform as well as the importance of continuous medium circulation to assess liver-mediated pro-drug activation and de-toxification.

3461 Abstract Withdrawn by Author.

3462 *In Vitro* Metabolism of Benzo[a]pyrene-7,8-Dihydrodiol and Dibenzo[def,p] Chrysene-11,12 Diol in Rodent and Human Hepatic Microsomes

D. Mehinagic[2], J. N. Smith[2], S. R. Crowell[1], N. C. Sadler[2], A. T. Wright[2] and R. A. Corley[2]. [1]*Genentech, South San Francisco, CA and* [2]*Pacific Northwest National Laboratory, Richland, WA.*

Polycyclic aromatic hydrocarbons (PAHs), many of which are carcinogenic, are contaminants that are ubiquitously found in the environment and often produced through natural and anthropogenic combustion of organic matter or petrochemical sources. The prototypic PAH, benzo[a]pyrene (B[a]P) and the highly carcinogenic dibenzo[def,p]chrysene (DBC) are metabolically activated by isoforms of the P450 enzyme superfamily producing benzo[a]pyrene-7,8-dihydrodiol (B[a]P-diol), dibenzo[def,p]chrysene-11,12 diol (DBC Diol). Each of these diols are further metabolized by P450s to highly reactive diol-epoxide metabolites that readily react with DNA. To complement prior *in vitro* metabolism studies with parent B[a]P and DBC, metabolism of B[a]P-diol and DBC-Diol were studied in hepatic microsomes from female B6129SF1/J mice, male Sprague-Dawley rats, and female humans. Activity-based protein profiling was also used to characterize the relative activities of P450s in each species. Michaelis-Menten kinetic parameters including affinity constants (KM, µM), maximum rates of metabolism (VMAX, nmol/min/mg microsomal protein), and rates of intrinsic clearance (CLINT, ml/min/kg body weight) were calculated from substrate depletion data. CLINT rates were also determined using a linear regression method. Clearances rates of B[a]P-Diol were highest in the mouse (266.10 ± 192.31 ml/min/kg body weight) and lowest for the human (50.766 ± 5.22 ml/min/kg body weight). Clearance rates of DBC-Diol were linear for the mouse and human, but saturable for the rat (64.31 ± 3.65 ml/min/kg body weight) over concentration ranges up to solubility limits. For both B[a]P-diol and DBC-diol the mouse had the highest clearance rates. Generally diol metabolite clearance is much greater than the parent compound clearances previously studied. Findings reported here provide a substantial basis for extension of PBPK models for B[a]P and DBC to include their diol metabolites. Funded by NIEHS Grant No. P42 ES016465.

3463 Hepatotoxicity Evaluation of Hydroethanolic Extract of a Medicinal Plant *C. sylvestris* (*salicaceae*), in Rat Liver

A. Z. Ameni[3], G. Schmeda- Hirschmann[2], K. A. Simon[1] and S. L. Gorniak[3]. [1]*Biological Sciences, Federal University of São Paulo, Diadema, Brazil;* [2]*Natural Products, University of Talca, Talca, Chile and* [3]*Pathology, University of São Paulo, São Paulo, Brazil.*
Sponsor: K. Welch.

Background: *Casearia sylvestris (Salicaceae)* is widely used as a medicinal plant in Brazil, where it catalogued by the Brazilian Unified Health System as a plant of interest for the Brazilian population with the purpose of treating inflammatory conditions, such as pain and gastrointestinal disorders based on the folk use. Although our previous studies with *C. sylvestris* did not revelead toxic effects in acute toxicity trial, as well as in systemic or immunotoxic evaluation in studies with repeated doses of 28 and 90 days, no toxicological evaluation concerned the extract fluid of *C. sylvestris* on liver have been reported. The liver is an important target of the toxicity of xenobiotics and oxidative stress. Therefore, the aim of this study was evaluate the effects of the hydroethanolic extract of *C. sylvestris* (HEC) on hepatic oxidative stress and on modulation of microssomal cytochrome P450 enzyme complex in rat liver. Methods: Seventeen adult male Wistar rats, 200-250 g, were divided into groups as follows: control (water), positive control Phenobarbital (120mg/kg) and HEC (240mg/kg). After six consecutive days of treatment it was evaluated the activity of antioxidant enzymes superoxide dismutase and catalase, gluthatione (reduced form GSH; oxidized form GSSG) and lipid peroxidation (concentration of thiobarbituric acid reactive substances) on liver homogenate. The effect of HEC on total cytochrome P450 content was assessed on the microsomal fraction rat liver. Results: The results obtained showed an alteration on the redox-status of glutathione with the GSH/GSSG ratio decreased in treated animals. Conclusions: Use of *C. sylvestris* concomitantly with other drugs, could present risk of drug interactions.

3464 Metabolite Profile Identification Using Alginate Supported 3D Culture of Primary Hepatocytes

J. M. Pedersen[2], J. Shim[2], E. L. LeCluyse[2], J. M. Macdonald[1], M. E. Andersen[2], H. J. Clewell[2] and M. Yoon[2]. [1]*The University of North Carolina, Chapel Hill, NC and* [2]*Center for Human Health Assessment, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Incorporating human metabolism kinetics in integrated testing approaches for non-animal based safety assessments is important for accurately predicting clearance and testing for metabolite-mediated toxicity. Despite recent advances in hepatocyte culture techniques, metabolic competence still decreases over time in culture and predicting *in vivo* metabolite profiles from *in vitro* data remains challenging. The aim of this project was to develop a large scale hepatic *in vitro* model that generates *in vivo*-relevant metabolite profiles in sufficient amounts for metabolite identification. Primary hepatocytes were cultured for 30 days under dynamic condition in three dimensional (3D) alginate hydrogel. Encapsulation procedures and culture conditions were optimized using 3D fluid dynamic modeling. The high cell-to-media ratio in the alginate bead culture is a key factor to obtain the desired yields of metabolites. Hepatocyte metabolic competence over time in culture was characterized by weekly 7-ethoxycoumarin (7-EC) and acetaminophen (APAP) incubations. Our alginate bead cultured hepatocytes maintained metabolic capacity and metabolite profiles for 7-EC and APAP during the first seven days in culture. A progressive decline in metabolic competence and shifting metabolite profiles were observed between days 14-28. However, when transferred to a flow based liver bioreactor after day 28, metabolic competence was restored to half of that observed in hepatocyte suspensions. We are now conducting experiments that include incubations of chemicals with high human exposure concerns, such as chlorinated tris and parabens, to produce relevant *in vivo* metabolites. In combination with *in silico* based prediction of metabolite bioactivity our bioreactor can provide a tool to screen for potentially active metabolites and provide key metabolism support for non-animal based toxicity testing. (Funding provided by American Chemistry Council Long-Range Research Initiative)

3465 Molecular Mechanism of Regulation of Drug Metabolizing Enzymes/Transporters *In Vivo*: Genomic Profiling and Proteomic Approaches

G. Taneja[2], W. Jiang[1], S. Maity[1], C. Coarfa[1], B. Moorthy[1] and R. Ghose[2]. [1]*Department of Pediatrics, Baylor College Of Medicine, Houston, TX and* [2]*Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX.*

Background: Disruption of drug metabolism/transport is impaired in many diseases, which is associated with activation of pro-inflammatory cytokines. Impairment in metabolism due to altered DME and transporter levels results in failure of therapy or adverse drug reactions, emphasizing the need to understand their regulation at a genomic level. Expression of DMEs & transporters is regulated via basal transcription factors and activation of nuclear receptors (PXR, CAR etc.). However the exact mechanisms are unknown. Objective: To determine genome-wide changes associated with up-regulation & down-regulation of DMEs & transporters. Methods: C57BL/6 mice were pre-treated with corn oil (CO) or pregnenolone-16alpha-carbonitrile [PCN (mPXR activator)] to induce DMEs, followed by saline (Sal) or lipopolysaccharide [LPS (bacterial endotoxin which activates pro-inflammatory cytokines)] to downregulate DMEs. Hepatic gene expression was evaluated using Illumina MouseWG-6 v2.0 expression beadchip. Data was normalized using lumi package and analyzed using R statistical system. Analysis of transcription factors was done using hypergeometric distribution ($p<0.05$). Results: 136 genes were significantly unregulated (UR) (~5 folds) with PCN and 1059 genes were significantly down regulated (DR) (~5 folds) with LPS as compared to CO/Sal. In PCN/LPS group, 1882 genes were differentially expressed (UR: 829, DR: 1053) as compared to CO/Sal. These differentially expressed genes include- phase 1 DMEs (Cyp2a, 2c, 3a11 etc.), phase 2 DMEs (ugt1a1, 1a5, 1a10 etc.), transporters (SLC01A4 etc.), cell signaling pathways (e.g. inflammatory pathways, oxidative phosphorylation etc.) and many more. Protein expression was further assessed by RPPA assay and preliminary analysis shows PCN treatment significantly upregulated cell signaling proteins (c-Fos, SOX9, annexins etc) by ~7 fold and LPS downregulated transcription factors (STAT3, p27, integrins etc) by ~10 fold as compared to CO/Sal. Conclusion: Novel regulators that are involved in both upregulation and downregulation of DMEs and transporters were identified. Ultimately, identification of these regulators will lead to development of targeted strategies to treat disorders due to impaired drug metabolism.

3466 Cardiac Micrornas as Biomarkers to Assess Doxorubicin-Induced Cardiotoxicity in Human Cardiomyocytes Derived from Pluripotent Stem Cells

X. Yang, W. Wang and L. Ren. *Systems Biology, US FDA/NCTR, Jefferson, AR.*

MicroRNAs (miRNAs), a novel class of non-coding RNAs of 22~24 nucleotides in length, are crucially involved in cardiac development and normal function. Some miRNAs are enriched in the heart tissue, and as such they may have value as more sensitive, specific, and mechanistic biomarkers of drug-induced cardiotoxicity. In addition, miRNAs have recently been detected in human body fluids and circulating miRNAs have been assessed as diagnostic biomarkers. Doxorubicin is a well-established chemotherapeutic agent routinely used for the treatment of malignant tumors; however, its cumulative drug-induced cardiac toxicity limits its long term use. To discover novel biomarkers of doxorubicin toxicity we have explored the use of human induced pluripotent stem cells (iPSC) differentiated into functional cardiomyocytes (CMs), an emerging *in vitro* model for drug safety testing. We examined changes in miRNAs profiles in response to doxorubicin exposure in both the cultured CMs and the cell-free supernatant following to different concentrations of doxorubicin for up to 1 day. Cells and cell-free supernatant samples (1h, 3h, 6h and 24h) were collected for miRNA and cardiac specific troponin I analysis. Our findings suggest that a majority of the differentially expressed cellular miRNAs were related to cell death, inflammatory response and tissue injury. Furthermore, cardiac miRNAs (miR-1 and miR-208) in the cell-free medium increased in response to drug-induced stress as early as 1h post-treatment, in the absence of overt injury, which was measured by TnI release. Results from this work have the potential to evaluate the utility of miRNAs as sensitive and specific biomarkers of drug-induced cardiac toxicity.

3467 Evaluation of microRNAs 122, 126 and 21 as Potential Biomarkers of Hepatic Microvascular Injury

P. Rana[2], H. Wu[2], L. Nelms[2] and K. Adkins[2]. [1]*Drug Safety R & D, Investigative Toxicology, Pfizer, Groton, CT and* [2]*Investigative Toxicology, Pfizer, Groton, CT.* Sponsor: P. Rana.

Antibody-drug conjugates (ADCs) that use the DNA-damaging agent calicheamicin as a cytotoxic payload, such as Mylotarg, are associated with hepatotoxicity that in some cases can progress to severe veno-occlusive disease. There are currently no early detection biomarkers available to determine patients at risk for progression to severe hepatoxicity. The objective of this study was to evaluate three microRNAs (miR-122, miR-126, and miR-21) as potential biomarkers for microvascular hepatotoxicity observed in monkeys treated with a calicheamicin ADC. This ADC contains a non-binding antibody that does not recognize any epitope in the monkey, and the same linker-payload employed in Mylotarg. The monkeys received either vehicle or ADC once every three weeks (Days 1, 22 and 43) and serum was collected for microRNA isolation at time points prior to the initiation of dosing (Day -7) and on Days 4, 11, 18, 25, 32, 42, 46, 53, and 63. The amounts of miR-122,-126, and -21 in the serum were determined by real-time quantitative PCR (qPCR). There was a higher amount of miR-122 observed in the ADC- treated animals when compared with controls at each time point evaluated after study initiation, with the greatest difference toward the end of the study (Day 35-50). For miR-126 there was a trend towards lower amounts observed in the ADC-treated monkeys for most of the time points evaluated after the first dose, and for miR-21, lower amounts were observed at all of the time points after the first dose in the ADC-treated animals. However, the magnitude of the differences observed was relatively small (≤7-fold), and there was a large amount of inter-animal variability observed in both control and ADC-treated animals at all of the time points evaluated in this study. In summary, there were ADC-related changes in the amount of miR-122, -21, and -126 detected in the serum following administration to cynomolgus monkeys; however additional work is needed to fully understand the utility of these miRNAs as biomarkers of hepatic microvascular injury.

3468 Serum miRNAs as Prognostic Indicators of Toxic Liver Injury

M. G. Permenter[1], B. C. McDyre[2] and D. L. Ippolito[3]. [1]*EXCET/US Army Center for Environmental Health Research, Ft. Detrick, MD;* [2]*ORISE/US Army Center for Environmental Health Research, Ft. Detrick, MD and* [3]*US Army Center for Environmental Health Research, Ft. Detrick, MD.* Sponsor: J. Lewis.

Exposure to toxic industrial chemicals resulting in liver injury is an occupational hazard for many workers, community members, and military personnel. Here we identify a panel of serum miRNAs corresponding with transcriptomic responses to toxic liver injury in the tissue. Rats were treated with 162 mg/kg of the liver injury inducing chemical 4, 4′-methylenedianiline (MDA) or vehicle control for 5 days by oral gavage, and serum and liver tissue were collected at necropsy. Liver sections were stained with hematoxylin and eosin. Serum miRNA expression was measured by next-generation sequencing. Gene expression in the liver was determined by microarray, and global serum and liver protein expression was determined by semi-quantitative mass spectrometry. MDA administration caused moderate liver fibrosis and hyperplasia. We identified 16 differentially expressed miRNA in treated rats (12 upregulated, including miR-182, miR-122-5p, and -3p; 4 downregulated, including miR-340-5p and miR-182; FDR<0.05). Transcriptomic analysis identified 506 differentially expressed genes in the liver. 13 miRNAs were predicted to target 100 unique differentially expressed genes in the liver. Global proteomics analysis identified 131 differentially expressed proteins in the liver and 53 in the serum. Of these, 7 miRNAs targeted 13 unique genes coding for differentially expressed liver tissue proteins and 3 miRNAs targeted 3 unique genes coding for serum proteins. In conclusion, we identified 16 miRNAs in the serum which are predicted to target and potentially regulate gene expression contributing to the progression of toxic liver injury. These 16 miRNAs are candidate serum biomarkers of toxic liver injury. Disclaimer: This research was supported in part by an appointment to the Postgraduate Research Participation Program at the USACEHR administered by ORISE through an interagency agreement between the US DOE and USAMRMC. Research was conducted in compliance with the Animal Welfare Act and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.

3469 High-Throughput, Purification-Free, Multiplexed Profiling of Circulating miRNA for Discovery, Validation, and Diagnostics

J. D. Tytell. *Firefly, Abcam, Inc, Cambridge, MA.* Sponsor: R. Church.

To address the needs for circulating miRNA biomarker validation, we developed the Multiplexed Circulating microRNA assay. This assay enables the detection of up to 68 microRNA targets per sample in 96-well format with readout on standard flow cytometers and analysis with an included bioinformatics software package. The Circulating microRNA assay combines particle-based multiplexing, using patented Firefly™ hydrogel particles, with single step RT-PCR signal amplification using universal primers. Thus, the Circulating microRNA assay leverages PCR sensitivity while eliminating the need for separate reverse transcription reactions and mitigating amplification biases introduced by target-specific qPCR. The Circulating microRNA assay can reliably detect as few as 1000 microRNA copies per sample with a linear dynamic range of ~5 logs, making the assay ideally suited for profiling in serum, plasma or urine. Furthermore, the ability to multiplex targets in each well eliminates the need to split valuable samples into multiple reactions. Results from the Circulating microRNA assay are displayed and interpreted using our included Firefly Analysis Workbench, which allows visualization, normalization, and export of experimental data with only a few mouse clicks. To aid discovery and validation of biomarkers, we have generated fixed panels for Oncology, Cardiology, Neurology, Immunology, and Liver Toxicology. These carefully curated panels include hemolysis markers to assess sample quality, as well as critical normalization factors. Here we present the data from several studies investigating circulating and tumor microRNA profiles using the Firefly Circulating microRNA Assay Fixed Panels. Together, this novel combination of bioinformatics tools and multiplexed, high-sensitivity assays enables rapid discovery and validation of microRNA biomarker signatures from fluid specimens.

3470 Serum miRNAs as Biomarkers of Nephrotoxicity in Female and Male Rats Fed a Diet Containing Melamine and Cyanuric Acid

C. S. Silva[2], C.-W. Chang[2], G. Gamboa da Costa[2] and L. Camacho[2]. [1]*Division of Biochemical Toxicology, NCTR/US FDA, Jefferson, AR and* [2]*Division of Bioinformatics and Biostatistics, NCTR/US FDA, Jefferson, AR.*

Circulating microRNAs (miRNAs) have been proposed as useful biomarkers of tissue injury and disease for multiple organs, including the kidney. We have shown previously that several serum miRNAs were affected by a 28-day combined dietary exposure to melamine (MEL) and cyanuric acid (CYA) in male NCTR F344 rats. The combination of these compounds is known to lead to the formation of crystals in the nephrons and consequent obstruction of renal tubules, which may result in acute kidney failure. In the current study, we expanded our investigation to female F344 rats. Similar to males, the miRNome of serum samples from females (n = 3-4/dose group) was screened using quantitative real-time RT-PCR (qRT-PCR) and several miRNAs were found to be differentially expressed (p-value < 0.05; fold-change ≥ 2.0) in the high dose group (240 ppm MEL & CYA) versus vehicle control. Eight of the 32 down-regulated miRNAs in females were common to those identified as down-regulated in the males. In addition, and similar to the findings in males, miR-128-3p was down-regulated in female serum from the 180 ppm MEL & CYA dose group. The screening data were validated by confirmatory qRT-PCR (n = 10-12/dose group); three additional dose groups (30, 60, 120 ppm MEL & CYA) were included to characterize better the dose-response. As previously described (Gamboa da Costa et al., 2012), kidney lesions in these animals were increased in the 180 and 240 ppm MEL & CYA groups; however, blood urea nitrogen (BUN) and serum creatinine were increased only in the 240 ppm MEL & CYA group. Our current results support the use of serum miRNAs as biomarkers of nephrotoxicity and reinforce the observation that miR-128-3p may be a more sensitive biomarker of kidney toxicity than BUN or serum creatinine in rats. IAG FDA 224-12-0003/NIEHS AES12013.

3471 Impact of Data Normalization and Hemolysis in the Quantification of Serum MicroRNAs

L. Camacho, C. S. Silva and C.-W. Chang. *US FDA/NCTR, Jefferson, AR.*

Circulating cell-free microRNAs (miRNAs) are promising biomarkers of disease and toxicity. Numerous publications suggest their superior specificity and/or sensitivity when compared with other traditionally used circulating biomarkers, for example, to assess nephrotoxicity (blood urea nitrogen and serum creatinine) or hepatotoxicity (alkaline phosphatase and alanine aminotransferase). The gold standard to quantifying miRNAs inbiofluids is quantitative real-time RT-PCR, a technique widely available and easy to use; however, several extrinsic factors can have a negative impact on the outcome of the study. One such factor is the choice of the normalizer miRNA to correct the data, e.g., for differences in template input; another factor is the contribution of hemolysis to the make-up of the serum miRNome. In the current study, we screened the serum miRNome of NCTR F344 rats (n=11-12/sex) and identified miR-342-3p as a stable serum miRNA in both sexes; the expression level of this miRNA was moderate, making it a suitable candidate for a normalizer. We further confirmed its stability using a larger sample size (n=65-70/sex). We then assessed the impact of hemolysis in this and other serum miRNAs by quantifying specific miRNAs in non-hemolyzed rat serum samples that had been spiked with increasing volumes of hemolyzed blood. As expected, the serum levels of miR-451-5p, an erythrocyte-enriched miRNA, significantly correlated with that of hemoglobin, while miR-23-3p, a miRNA known to be unaffected by hemolysis, did not. The levels of miR-342-3p were also not affected by the hemolysis level. We conducted a similar correlation study using serum samples with different levels of hemolysis, but in which each sample originated from a different animal. No correlation was found between the hemoglobin levels and the levels of miRNAs, including miR-451-5p. These data suggest that, although hemolysis can impact the levels of certain miRNAs in serum, the biological variability between animals may outweigh this effect.

3472 Comprehensive MicroRNA Profiling in Acetaminophen Toxicity Identifies Novel Circulating Biomarkers for Human Liver and Kidney Injury

A. D. Vliegenthart[3], J. M. Schaffer[2], J. I. Clarke[4], L. E. Peeters[3], A. Caporali[3], D. N. Bateman[3], D. M. Wood[1], P. I. Dargan[1], N. C. Henderson[3], D. J. Webb[3], J. Sharkey[4], D. J. Antoine[4], B. K. Park[4], M. A. Bailey[3], E. Lader[2] , K. J. Simpson[3] and J. W. Dear[3]. [1]*King's College London, London, United Kingdom;* [2]*Qiagen, Fredrick, MD;* [3]*University of Edinburgh, Edinburgh, United Kingdom and* [4]*University of Liverpool, Liverpool, United Kingdom.*

The objective of the study was to identify microRNA (miRNA) biomarkers of drug-induced liver and kidney injury by profiling the circulating miRNome in patients with acetaminophen overdose. Plasma miRNAs were quantified in age- and sex-matched acetaminophen overdose patients with (N=27) and without (N=27) organ injury (APAP-TOX and APAP-no TOX, respectively). Classifier miRNAs were tested in a separate test cohort (N=81). miRNA specificity was determined in non-acetaminophen liver injury and murine toxicity models. Sensitivity, in comparison with the current biomarker ALT, was tested by stratification of patients at hospital presentation (N=67). From 1809 miRNAs, 75 were 3-fold or more increased and 46 were 3-fold or more decreased with APAP-TOX. A 16 miRNA classifier model accurately diagnosed APAP-TOX in the test cohort. The miRNAs with the largest fold increase (miR-122-5p, miR-885-5p and miR-151a-3p) and highest ranked miRNA from the classifier model (miR-382-5p) accurately reported non-acetaminophen liver injury and were unaffected by kidney injury. A panel of novel miRNAs were associated with kidney dysfunction. miR-122-5p was more sensitive than ALT for the diagnosis of liver injury at hospital presentation, especially when combined with miR-483-3p. Profiling of acetaminophen toxicity identified multiple miRNAs that report acute liver injury and potential biomarkers of drug-induced kidney injury.

3473 Serum MicroRNA Biomarkers of Acute Pancreatic Injury in the Dog

R. Rouse, B. Rosenzweig, L. Xu, S. Stewart, A. Chockalingam, A. Knapton and K. Thompson. *Division of Applied Regulatory Science, US FDA, Silver Spring, MD.*

Following identification and characterization of microRNA biomarkers of acute pancreatic injury in rats and mice, studies were designed to characterize the response of the pancreas-enriched microRNAs miR-216a, miR-216b, miR-217, and miR-375 and microRNAs enriched in brain or liver in a canine model of caerulein-induced acute pancreatic injury. Studies were run with one set of four male beagles 6 to 8 months of age to pilot the use of subcutaneous caerulein, for assay development, and to test the time course and dose response of microRNAs of interest compared to gold standard assays. A caerulein dose range finding study with 1 dog per dose was run first, followed by equivalent dosing of all dogs at 3 progressively lower doses, with 4-5 weeks between dosing. Serial sampling was taken pre-treatment and at 1, 3, 6, 12, 24, 48, 72 hours, and 1 week post-treatment. The four pancreas-enriched microRNAs showed a dose-related increase in serum levels that was detectable at 1 hr, peaked at 3-6 hours, and returned to near baseline levels by 24 to 48 hours post-treatment for total caerulein doses of 5, 25, 50, 100, or 150 µg/kg. Serum amylase demonstrated similar dose response and temporal response to the microRNAs, but with a reduced dynamic range of response and a slower return to baseline (48 to 72 hours). . Serum pancreatic specific lipase did not consistently show a dose response, was more influenced by individual variation, and took longer to return to baseline at higher exposures. Follow on experiments using naïve groups of 4 dogs should help to confirm responses and link biomarker level changes to histologically identifiable changes in acinar and islet cell morphology.

3474 Integrated Mrna and Microrna Sequencing Identifies Microrna-mRNA Regulatory Network in a Rat Model of Acute Pancreatic Injury

R. Rouse[1], B. Rosenzweig[1] and Z. Li[2]. [1]*Division of Applied Regulatory Science, US FDA, Silver Spring, MD and* [2]*Division of Applied Regulatory Science, US FDA, Silver Spring, MD.*

To study molecular signaling pathways associated with pathological changes during pancreatitis, large scale microRNA and mRNA co-sequencing was performed on pancreas tissues in a rat model of acute pancreatitis. During a previous study charactering pancreatic enriched microRNAs as biomarkers of injury, rats were given either caerulein (50µg/kg) or saline via repeated intraperitoneal injection. At each of five

time points following the final injection (1, 3, 6, 24, & 48 hours), pancreatic tissue obtained from six caerulein- and six saline-treated rats was subjected to microRNA and mRNA sequencing as well as histopathology. Next generation sequencing (NGS) identified 206 microRNAs and 12322 mRNAs that were differentially expressed (DE) between treated and control samples in at least one time point (FDR <= 0.05). Time series analysis of these DE genes revealed 4 distinct microRNA response profiles and 12 distinct mRNA response profiles during pancreatic injury. An integrated analysis combining microRNA target prediction and microRNA/mRNA anti-correlation identified 27502 microRNA-mRNA pairs that formed an entangled regulatory network. Pathway analysis suggested this network primarily affects important cellular processes like cellular proliferation, cell cycle, cell movement, and lipid metabolism involved in response to injury. A closer investigation of candidate microRNA biomarkers of pancreatic injury (miR-216a, miR-216b, miR-217, miR-375) was initiated to identify potential mechanisms of action and therefore mechanistic anchors for these candidate biomarkers.

PS 3475 MicroRNA Profiling of Plasma from B6C3F1 Mice Treated with Doxorubicin

T. Han, C. L. Moland, V. D. Desai and J. C. Fuscoe. *Division of Systems Biology, National Center for Toxicological Research, Jefferson, AR.*

Doxorubicin (DOX) is an anti-cancer drug often used to treat leukemia, lymphoma, and many types of carcinoma. However, it can cause irreversible cardiotoxicity in a dose-related manner in cancer patients. The current cardiac markers, troponin T/I, can only be detected in plasma after the cardiac injury occurred. There is a lack of biomarkers for predicting the risk of cardiac damage. Growing evidence suggests microRNAs (miRNAs) may play a role in susceptibility to certain toxicities and adverse drug reactions. They have emerged as promising biomarkers of cardiovascular pathologies and therapeutic response. In this study, male B6C3F1 mice were given a weekly dose of 3 mg/kg DOX, or an equivalent volume of saline (SAL) via tail vein for 2, 3, 4, 6, and 8 weeks, resulting in cumulative DOX doses of 6, 9, 12, 18, and 24 mg/kg, respectively. Mice were sacrificed a week after the last dose. Increase of cardiac troponin T in plasma indicated cardiac cell injury following exposures to 18 mg/kg DOX and higher, and microscopic examination of heart revealed the presence of cardiac lesions at 24 mg/kg. Agilent mouse miRNA microarrays consisting of 1,881 unique miRNAs were used to evaluate miRNA expression in the plasma of these mice. The expression of 53, 17, 10, 56, and 64 miRNAs was altered at cumulative DOX doses of 6, 9, 12, 18, and 24 mg/kg, respectively, compared to SAL controls (t-test, $p<0.05$). Expression of pro-apoptotic miRNA-34a was elevated dramatically by 5.7- to 11.8-fold, at 9 mg/kg and higher cumulative DOX doses compared to concurrent SAL-treated controls and the increase was significant only at 9 and 18 mg/kg DOX doses. Ingenuity Pathway Analysis showed these differentially expressed miRNAs were mainly involved in cellular development, cellular growth and proliferation, and cell death and survival. Expression of miRNAs related to cardiac hypertrophy was also perturbed at 6, 18, and 24 mg/kg DOX. These findings may lead to the identification of early predictive biomarkers of cardiac injury for application in clinical therapies.

PS 3476 Microrna Profiling Identifies Potential Biomarkers of Hepatobiliary Injury Following Exposure to Several Toxicants in the Rat

R. J. Church[5], M. Pavkovic[2], M. Otieno[4], H. Ellinger-Ziegelbauer[1], B. Riefke[1], J. E. McDuffie[3], B. Singh[4], M. Sonee[4], L. Hall[4], P. B. Watkins[5] and A. H. Harrill[5,6]. *[1]Bayer Pharma AG, Wuppertal, Germany; [2]Harvard Medical School, Boston, MA; [3]Janssen Research & Development, San Diego, CA; [4]Janssen Research & Development, Spring House, PA; [5]The Hamner-UNC Institute for Drug Safety Sciences, Research Triangle Park, NC and [6]University of Arkansas Medical School, Little Rock, AR.*

MicroRNAs (miRNAs) are promising biomarkers of drug-induced liver injury; however, to date, candidate identification has mostly been limited to profiling in acetaminophen-induced toxicity. In this study, plasma miRNA alterations were quantified in male Sprague Dawley rats orally administered either a single dose of α-naphthylisothiocyanate (ANIT; 50 mg/kg) or daily doses of FP004BA (100 mg/kg/day) or respective vehicles, and in male Wistar rats orally administered methapyrilene (MPy; 30 and 80 mg/kg) or vehicle. Results were phenotypically anchored to the temporal progression of hepatobiliary injury and compensatory biliary hyperplasia which these compounds are known to elicit. Rats sacrificed 6, 24, 48, 72, 120, or 168 hours (h) post dose with ANIT (n=6/treatment/time point) displayed significant hepatobiliary injury from 24h to-72h post-dosing; hyperplasia was observed from 48h post-dose through study termination. Rats administered FP004BA, a Bayer Pharma AG proprietary compound, for 1, 3, or 14 consecutive days (d; n=6/treatment/time point), developed progressive hepatobiliary injury following 1 and 3d, biliary hyperplasia following 3 and 14d, and fibrosis following 14d. MPy- and vehicle-treated rats (n=6/treatment/time point) were sacrificed following 3, 7, or 14 daily doses of compound; a recovery group was sacrificed 10 days following 14 consecutive administrations of MPy or vehicle. Histopathological findings included periportal necrosis, inflammation and biliary hyperplasia; all findings increased dose and time dependently. Plasma miRNA profiling revealed that approximately 60-120 miRNAs were altered in blood, depending on the chosen selection thresholds, in each study. 25 miRNAs were commonly altered by the three toxicants examined in this investigation, independent of time and directionality. In addition to miR-122-5p and -192-5p, miR-802-5p, -200a-3p, -30a-5p, and -30e-3p were consistently elevated during hepatobiliary injury caused by all toxicants, suggesting that these species may be potential biomarker candidates for hepatobiliary injury.

PS 3477 Evaluation of miR-217 and miR-375 as Biomarkers of Pancreatic Injury

M. Tirmenstein[2], J. Calvano[2], C. Hixson[2], G. Edwards[2], B. Baumgart[1], C. Korgaonkar[1] and R. Mangipudy[2]. *[1]Drug Safety Evaluation, Bristol-Myers Squibb, Mt. Vernon, IN and [2]Drug Safety Evaluation, Bristol-Myers Squibb, New Brunswick, NJ.*

Pancreatic injury in preclinical toxicity studies is primarily detected through histopathological changes and conventional serum biomarkers such as amylase/lipase. However, amylase/lipase have a short half-life and are markers of pancreatic acinar not islet cell injury. We investigated whether circulating microRNA (miR) levels that are enriched in acinar cells (miR-217) or islet cells (miR-375) could serve as markers of pancreatic injury. Rats were treated with a single ip dose of either vehicle, the pancreatic acinar cell toxicant--caerulein (0.05 mg/kg), or the ß-cell toxicant--streptozotocin (STZ) (150 mg/kg). Rats were necropsied at 4, 24, and 48 hrs and pancreas, liver, heart, kidney and skeletal muscle were collected and analyzed for histopathology. Blood was collected at necropsy and processed to serum for amylase/lipase enzymatic determinations and miR qPCR analysis. Caerulein induced degeneration/necrosis of acinar cells after 4 hrs that persisted after 24 and 48 hrs, and increased amylase/lipase levels at 4 hrs but not at 24 or 48 hrs. Increases in miR-217 (100-1000x controls) persisted for 48 hrs following caerulein and only occurred in rats with evidence of acinar cell damage. STZ induced islet cell necrosis after 4 hrs followed by atrophy at 24 and 48 hrs. STZ did not induce significant increases in either amylase or lipase but did induce increases in miR-375 levels at 4 (90x controls) and 24 hrs (5x controls). No significant increases in miR-375 were observed in caerulein-treated or miR-217 in STZ-treated rats. Our results indicate that used together, miR-217 and miR-375 represent promising biomarkers of exocrine and endocrine pancreatic injury in rats.

PS 3478 Relative Tissue Distribution of miRNAs in the Cynomolgus Monkey

J. Calvano, X. Zhang, H. Jonassen, R. Mangipudy and M. Tirmenstein. *Drug Safety Evaluation, Bristol Myers Squibb, New Brunswick, NJ.*

MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression. Several miRs are currently being evaluated as potential circulating markers of target organ toxicity due to their stability, tissue specific distribution, and conserved expression across multiple species. Only a few studies have reported the relative expression of miRs in normal tissues with most of these being conducted in rodents. Limited information exists for tissue distribution of miRs in other species commonly used in safety assessment. We examined the relative expression of miRs in normal cynomolgus monkey tissue using human TLDA card arrays. Tissues examined included the heart (left ventricle and aortic valve), skeletal muscle (soleus and quadricep femoris), pancreas, liver, kidney, and eye (retina and cornea). Examination of the heart tissue revealed enrichment of 4 miRs in cardiac valves (miRs-320, 197, 125b, 342-5p) and 8 in the left ventricle (miR-133a/b, 1, 208b, 302a, 499-5p, 27b, 142-5p) compared to the other tissues. MiR-208 was specifically expressed in the heart where it was detected in both the left ventricle and aortic valve. In the skeletal muscle, miR-208b, 302a and 499-5p were detected only in the soleus while the expression of miRs 133a/b, 1 and 342-5p were enriched in both the soleus and quadricep femoris muscles. In the pancreas, miRs-216a/b, 217, 375 and 148a were highly enriched compared to the other tissues. MiR-192, 194 and 20a were highly enriched in the liver but only miR-122 was specifically expressed in the liver. No retina or cornea specific miRs were detected. Three miRs (miR-192, 194 and 215) were enriched in the

kidney compared to other tissues. In general, miR tissue distribution was similar in the cynomolgus monkey compared to the rat. Assessing such cross-species differences is an important step in establishing the translational value of miRNAs as biomarkers of target organ toxicity in relevant animal species used in safety assessment and in humans.

3479 Evaluation of the Liver-specific MicroRNA, MiR-122, as a Marker of *In Vitro* Hepatocyte Toxicity in Liver Slices and Cultured Rat Hepatocytes

S. Shi[2], J. Calvano[2], X. Zhang[2], V. Ly[1], B. Brock[1], R. Mangipudy[2] and M. Tirmenstein[2]. [1]*Biotransformation, Bristol-Myers Squibb, Lawrenceville, NJ and* [2]*Drug Safety Evaluation, Bristol-Myers Squibb, New Brunswick, NJ.*

MicroRNAs (miRNA) are small single stranded non-coding RNA molecules that regulate gene expression at the post-transcriptional level. MiR-122 is specifically expressed in the liver and has been postulated to be involved in maintaining hepatocyte differentiation. Recently, circulating miR-122 levels have been used as a biomarker of liver toxicity in several different species including rats, dogs, and humans. We examined whether miR-122 release into extracellular media could also be used as a measure of cytotoxicity *in vitro* with cultured cryopreserved rat hepatocytes and in rat liver slices. MiR-122 levels were easily detected in both rat liver slices and in cryopreserved rat hepatocytes using qPCR techniques. MiR-122 levels were highly expressed in cryopreserved rat hepatocytes but its levels significantly decreased after 2 days in culture. The decline of intracellular miR-122 level was associated with the decrease of some rat cytochrome 450 enzymes (cyp3a2, cyp2d2 and cyp2e1). In contrast miR-122 levels were maintained over time in liver slices. Upon treatment with the toxicant, precocene, an increase of miR-122 release and decrease of intracellular miR-122 were observed at both 24 hours and 48 hours post exposure in rat liver slice incubations. The effects were moderate at 24 hours but much more significant (> 10 fold) at 48 hours. Following incubations with precocene in rat liver slices, miR-122 levels in the extracellular media increased which correlated with increases in LDH release. Our results indicate that miR-122 release is a potentially sensitive marker for assessing hepatocyte toxicity *in vitro*.

3480 Inotuzumab Ozogamicin-Induced Release of miRNA from Human Liver Sinusoidal Endothelial Cells

H. Wu[1], C. Doshna[1], L. Nelms[1], E. Rubitski[1], C. Davis[2] and K. Adkins[1]. [1]*Pfizer, Groton, CT and* [2]*Pfizer, San Diego, CA.*

Inotuzumab ozogamicin is an antibody-drug conjugate (ADC) composed of a monoclonal antibody targeting CD22 linked to a cytotoxic agent, calicheamicin, in development for the treatment of acute lymphocytic leukemia. Inotuzumab ozogamicin has been observed to cause veno-occlusive disease (VOD) in 1-5% of subjects in clinical studies. VOD is believed to result from damage to liver sinusoidal endothelial cells (LSECs). The objective of this study was to identify microRNAs (miRNAs) that are associated with inotuzumab ozogamicin-induced cytotoxicity in LSECs. MiRNA isolated from human liver tissue, hepatocytes, and LSECs, was assayed for 8 miRNAs. LSECs were then treated with inotuzumab ozogamicin, and miRNA amounts in the cell media were determined and evaluated for correlation with cytotoxicity, and relative relationship with DNA damage. MiRNA amounts were also evaluated in human serum samples from people with liver injury. All 8 miRNAs were detected in human liver tissue, hepatocytes, and LSECs, with 2 miRNAs at higher amounts in LSECs, and 3 miRNAs at higher amounts in hepatocytes. In primary human LSECs, the 2 miRNAs that had higher expression in LSECs, were increased at 24 hrs following inotuzumab ozogamicin treatment, whereas the 3 miRNAs that were higher in hepatocytes showed earlier increase at 4 hrs. In contrast, inotuzumab ozogamicin-induced DNA damage was detected as early as 1 hr after treatment. The levels of 4 out of these 5 miRNAs were significantly correlated with changes in ATP content ($p<0.05$, Pearson's correlation). Two out of the 3 miRNAs that were higher in hepatocytes were increased in serum samples from humans with liver injury evidenced by increased ALT ($p<0.05$, student t-test), whereas there was no significant increase observed in the 2 miRNAs that were higher in LSECs. Together, these data demonstrate that inotuzumab ozogamicin induces miRNA release following injury to primary human LSECs, and suggests that the miRNAs with higher expression in LSECs may be more specific to liver endothelial cell injury than general hepatotoxicity.

3481 Development and Qualification of Assays to Detect Circulating miR-122 in Rat and Human Serum to Support Preclinical and Clinical Studies

W. R. Proctor[2], K. S. Rajapaksa[2], J. Vogt[2], M. Tseng[2], J. Tarrant[2], D. Sheinson[2], S. Lewin-Koh[2] and D. Misner[2]. [1]*Nonclinical Biostatistics, Genentech, Inc., South San Francisco, CA and* [2]*Safety Assessment, Genentech, Inc., South San Francisco, CA.*

MicroRNAs (miRNAs) are small, double-stranded, non-coding RNA molecules approximately 18-25 nucleotides in length in their mature form. Due to their relative stability in circulation, tissue enrichment, and dynamic range of detection, miRNAs are being explored extensively for new biomarkers of disease, drug efficacy, and safety. miRNA-122 (miR-122) is highly expressed in hepatocytes and enriched approximately 10,000-fold in the liver relative to other organs. Increased levels of miR-122 in serum or plasma have been associated with drug-induced liver injury, viral hepatitis, and autoimmune hepatitis. As such, we have begun to evaluate circulating levels of miR-122 as an exploratory biomarker of liver injury in preclinical and clinical studies. Assays to detect miR-122 in human and rat serum were developed using qRT-PCR based detection on the Fluidigm Biomark HD platform with commercially available reagents and kits with minor modifications. Assay linearity, dynamic range, precision, and accuracy were assessed accordingly as part of a fit-for-purpose qualification. Circulating levels of miR-122 were measured in serum from healthy human volunteers (N=92) and naive or vehicle treated Sprague Dawley rats (N=71). As there is no consensus on best practices for normalization of circulating miRNAs, serum miR-122 levels were reported relative to either volume of sample, the expression of exogenous spike-in control (miR-Cel-39), or to the average expression of a panel of potential endogenous control miRNAs in circulation. Lastly, we evaluated miR-122 levels in rat serum samples from preclinical studies where liver injury was observed microscopically and in human serum from patients with abnormal/elevated liver enzymes. The biological variability of miR-122, the effects of different normalization approaches, and correlations to liver transaminase levels and/or liver injury will be presented. In conclusion, we have developed robust assays to quantify the relative expression of miR-122 in rat and human serum and are continuing to evaluate this exploratory biomarker in both the preclinical and clinical settings.

3482 Effects of Fiber Size of Single-Walled Carbon Nanotubes on Pulmonary Toxicity

K. Fujita[1,2], M. Fukuda[2], S. Endoh[2], J. Maru[2], H. Kato[1,2], A. Nakamura[1], N. Shinohara[1,2], K. Uchino[2] and K. Honda[1,2]. [1]*National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan and* [2]*Technology Research Association for Single Wall Carbon Nanotubes, Tsukuba, Japan.*

To elucidate the effect of fiber size on the pulmonary toxicity of single-walled carbon nanotubes (SWCNTs), we conducted a rat intratracheal instillation test using two types of dispersed SWCNTs, namely relatively thin bundles with short linear shapes (CNT-1), and thick bundles with long linear shapes (CNT-2). Total protein levels, MIP-1α expression, cell counts in bronchoalveolar lavage fluid (BALF), and histopathological examinations revealed that CNT-1 caused pulmonary inflammation and slower recovery, and that CNT-2 elicited acute lung inflammation shortly after their instillation. DNA microarray demonstrated that CNT-1-induced genes were strongly associated with inflammatory responses, cell proliferation, and immune system processes at 7 or 30 d post-instillation. Meanwhile, numerous genes were significantly upregulated or downregulated by CNT-2 at 1 d post-instillation. *In vitro* cell-based assays, CNT-1 and CNT-2 SWCNTs were phagocytized by NR8383 rat alveolar macrophages. CNT-2 treatment induced cell growth inhibition, reactive oxygen species production, MIP-1α expression, and several genes involved in response to stimulus, whereas CNT-1 treatment did not exert a significant impact in these regards. These results suggest that SWCNTs formed as relatively thin bundles with short linear shapes elicited delayed pulmonary inflammation with slower recovery. In contrast, SWCNTs with a relatively thick bundle and long linear shapes sensitively induced cellular response in alveolar macrophages and elicited acute lung inflammation shortly after inhalation. We conclude that the pulmonary toxicity of SWCNTs is closely associated with the fiber size of the bundles. These physical parameters are useful for risk assessment and management of SWCNTs. This study is based on results obtained from a project commissioned by the New Energy and Industrial Technology Development Organization (NEDO), Japan.

3483 Differential Analysis of the Effects Induced by Cellular Exposure to Pristine and Acid Treated Single Walled Carbon Nanotubes

R. Eldawud[2], A. Wagner[2], C. Dong[2], Y. Rojanasakul[2] and C. Dinu[2]. [1]*Basic Pharmaceutical Sciences, West Virginia University, Morgantown, WV and* [2]*Chemical Engineering, West Virginia University, Morgantown, WV.*

Despite the unique properties of single walled carbon nanotubes (SWCNTs), their implementation in a variety of biomedical and industrial applications had been hindered by many concerns associated with potential risks imposed by their exposure. Previous reports have shown that CNTs toxicity can be attributed to their length, surface chemistry, aggregation or metal impurities, however, no systematic investigation exists that correlates changes in the cellular dynamics with CNTs properties. Herein, we provide a comprehensive analysis of the induced cyto- and genotoxic effects associated with human lung cells exposure to pristine and acid treated SWCNTs. Specifically, by employing an electrical cell impedance sensing (ECIS) platform, we measured changes in the cellular behavior, attachment and cell-cell interactions as well as the overall cellular fate upon exposure to SWCNTs with different properties. We further complemented our real-time studies with conventional microscopy and flow cytometry techniques to derive structure function relationships that correlate the changes in the cellular dynamics at a molecular level with the SWCNTs' physico-chemical properties. Our results showed functional differences in the cellular behavior based on the nanotube's length, surface chemistry, purity and agglomeration state. Specifically, while all SWCNTs samples showed significant reduction in cellular viability, proliferation and migration, exposure to pristine SWCNTs lead to significant generation of ROS, the largest reduction in cellular migration and a cell cycle arrest at the G1/S phase. In contrast, exposure to the acid treated SWCNTs did not lead to ROS generation or major effects on cellular migration, however; it lead to more significant changes in the expression of structural and adhesion proteins and a higher percentage of cells arrested at the G2/M phase. Our results provide mechanistic insights into the induced cyto- and genotoxic effects associated with SWCNTs exposure as a function of their physical and chemical properties and further help define novel means to use electronic platforms and single cells as biosensors to assess the toxicity of different types of nanomaterials in real time.

3484 Detection of Transcriptomic Signature for a Senescence-Associated Secretory Phenotype (SASP) in Human Peritoneal Mesothelial Cells Exposed to Multiwalled Carbon Nanotubes

S. M. Reamon-Buettner, A. Hiemisch, I. Voepel and C. Ziemann. *In Vitro and Mechanistic Toxicology, Fraunhofer-ITEM, Hannover, Germany.* Sponsor: C. Dasenbrock.

Multiwalled carbon nanotubes (MWCNTs) have been shown in *in vivo* studies to cause mesothelioma, a tumor frequently associated with asbestos exposure. For instance, intraperitoneal treatment in rats with a tailor-made MWCNT (e.g. 'MWCNT A', geometric mean length: 8.57 µm, and diameter: 0.085 µm of WHO fibers weighted by mass) led to high-tumor incidence and early fatality (Rittinghausen et al. 2014 Part Fibre Toxicol 11:59). To identify molecular cues leading to MWCNT-induced mesothelioma, we carried out a parallel *in vitro* study on the same MWCNT using classical and molecular genotoxicity approaches in primary human peritoneal mesothelial LP9 cells. Findings included markers for cellular senescence, e.g. inhibition of cell division, nuclear fragmentation, chromatin condensation, senescence-associated heterochromatin foci (SAHFs), pan-nuclear staining with an anti-γH2AX antibody, and enlarged cells, being positive for senescence-associated β-galactosidase activity. Using microarray technology, we also analyzed MWCNT-treated (3 µg/cm2, 24 h incubation) LP9 cells, in which amosite asbestos served as positive, while milled MWCNT as material control. We found many differentially regulated genes in MWCNT A-treated cells, which were many-folds higher up- or downregulated, than in cells exposed to amosite or milled MWCNT. There were common biomarkers in MWCNT A and amosite, but also exclusive ones. Notably, expression of genes associated with senescence-associated secretory phenotype (SASP) was differentially regulated especially in MWCNT A, but not in asbestos- or milled MWCNT-treated LP9 cells. The detection of SASP signature (chemokines, cytokines, metalloproteases, and growth factors) supports our previous results to suggest for a potential role of cellular senescence in mesothelioma development after exposure to certain long and straight MWCNT.

3485 Functional and Molecular Responses to Inhalation of MWCNT From the Perspective of Occupationally-Relevant Depositions

V. K. Kodali[4], P. C. Zeidler-Erdely[4], T. Eye[4], S. Bilgesu[4], L. Bishop[4], S. Tugendreich[1], S. Shah[1], M. Campen[2], M. Aragon[2], M. L. Kashon[4], J. K. Gu[4], L. Battelli[4], J. L. Cumpston[4], A. Cumpston[4], W. McKinney[4], D. Frazer[4], T.-h. B. Chen[4], V. Castranova[3], R. Mercer[4] and A. Erdely[4]. [1]*QIAGEN, Redwood City, CA;* [2]*University of New Mexico, Albuquerque, NM;* [3]*West Virginia University, Morgantown, WV and* [4]*Health Effects Laboratory Division, National Institute for Occupational Safety & Health, Morgantown, WV.*

In order to assess the toxic response to occupationally-relevant depositions of multi-walled carbon nanotubes (MWCNTs), a dose- and time-dependent 4-week inhalation exposure (0.5-5 mg/m3) to MWNCTs (Mitsui-7) was conducted to model a worker exposed to 76 (40 µg alveolar deposition), 7.6 (4 µg) and 0.76 (0.4 µg) years at average inhalable workplace concentrations of 10.6 µg/m3. Mice were sacrificed at 0, 28, and 84 d post-exposure with lung, liver, and aorta collected for microarray-based gene expression profiling analyzed in conjunction with alterations in lavage proteins, alveolar macrophage function, histopathology, extrathoracic translocation, and systemic effects. Differentially expressed genes, upstream transcriptional regulators, and responses corresponding to inflammation/immune function and pathological changes (e.g. fibrosis) were persistent in the high dose but transient in the middle dose. The responses reflected the 58 lavage proteins measured and morphometric analysis of alveolar fibrosis which was increased in the high dose only. Similarly, isolated alveolar macrophages challenged with LPS (1 ug/ml) *ex vivo* had enhanced cytokine production that was sustained only in the high dose. At all doses, MWCNT translocated to extrathoracic organs indicating a dependence on physicochemical properties instead of a specific molecular mechanism. Analysis of systemic effects showed minimal to no changes in liver or aorta transcriptomics, 58 analyzed plasma proteins, or liver and renal histopathology. Endothelial cells challenged with plasma collected from the high dose group showed enhanced expression of adhesion molecules. In summary, altered molecular pathways, histopathology changes, and systemic effects occur primarily at depositions (or dose rates) predicted to be significantly higher than what was measured in 8 MWCNT workplaces.

3486 Toxicity Assessment and Bioaccumulation in Zebrafish Embryos Exposed to Carbon Nanotubes Suspended in Pluronic® F-108

R. Wang[4], A. N. Meredith[5], M. Lee[4], D. Deutsch[4], L. Miadzvedskaya[4], E. Braun[4], P. Pantano[4], S. Harper[5] and R. Draper[4]. [1]*Biological Sciences, University of Texas at Dallas, Richardson, TX;* [2]*Biological Sciences and Chemistry, University of Texas at Dallas, Richardson, TX;* [3]*Biological Sciens and Chemistry, University of Texas at Dallas, Richardson, TX;* [4]*Chemistry, University of Texas at Dallas, Richardson, TX and* [5]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Carbon nanotubes (CNTs) are often suspended in Pluronic® surfactants by sonication, which may confound toxicity studies because sonication of surfactants can create degradation products that are toxic to mammalian cells. Here we present a toxicity assessment of Pluronic® F-108 with and without suspended CNTs using embryonic zebrafish as an *in vivo* model. Pluronic® sonolytic degradation products were toxic to zebrafish embryos just as they were to mammalian cells. When the toxic Pluronic® fragments were removed, there was little effect of pristine multi-walled CNTs (pMWNTs), carboxylated MWNTs (cMWNTs), or pristine single-walled carbon nanotubes (pSWNTs) on embryo viability and development, even at high concentrations. A gel electrophoretic method coupled with Raman imaging was developed to measure the bioaccumulation of CNTs by zebrafish embryos, and dose dependent uptake of CNTs was observed. These data indicate that embryos accumulate pMWNTs, cMWNTs, and pSWNTs yet there is very little embryo toxicity.

3487 Lack of Genotoxicity in the Comet and Micronucleus Assays of Graphistrength© C100 Multiwalled Carbon Nanotubes (MWCNT) After A 90-Day Nose-Only Inhalation Exposure of Rats

J.-F. Régnier[5], S. Simar[4], F. Nesslany[4], E. Dony[2], D. Pothmann[3] and V. Shultz[1]. *[1]Arkema Inc., King of Prussia, PA; [2]Harlan Cytotest Cell Research GmbH, Rossdorf, Germany; [3]Harlan Laboratories Ltd, Itingen, Switzerland; [4]Laboratoire de Toxicologie Génétique, Institut Pasteur de Lille, Lille, France and [5]Toxicology and Environment, Arkema France, Colombes, France.*

Graphistrength© C100 provides superior electrical and mechanical properties for various applications. Graphistrength© C100 is formed of MWCNT (ca. 12 walls, outer mean diameter ca. 12 nm, length ca. 1 µm) agglomerated in particles with a granulometry centered on 400 µm. A general feature of MWCNT after inhalation or intratracheal exposures is the induction of an inflammatory reaction in the lungs sometimes associated with local genotoxic effects. Most of the *in vitro* and *in vivo* genotoxicity data available on Graphistrength© C100 are negative. However, a weak DNA damage activity in the *in vitro* and *in vivo* FPG-modified Comet assays and a weak clastogenic effect in the *in vitro* micronucleus test were reported. To evaluate the genotoxic potential of Graphistrength© C100 in the cells at the site of contact and those distant from it, Wistar rats were exposed by nose-only inhalation (6h/d, 5d/week) for 13 weeks to target concentrations of 0.05, 0.25 and 5.0 mg/m3 air of a respirable aerosol (MMAD < 3 µm). Twenty-four hours post-exposure, chromosomal aberrations in the bone marrow cells of males and females were evaluated by the micronucleus test (OECD TG 474) and DNA damage in the lung, kidney and liver cells of males were assessed by both the standard and the human 8-oxoguanine DNA N-glycosylase 1 (hOGG1)-modified comet assay (OECD TG 489). Broncho-alveolar lavage fluid (BALF) was also collected and analyzed for inflammatory parameters. Neither increase in the number of micronucleated polychromatic erythrocytes nor increase in percent DNA damage were observed at any concentration whereas an inflammatory lung reaction and the release of inflammatory factors in the BALF were observed in all rats exposed to 5.0 mg/m3 (see companion poster of the same author for details). Therefore, Graphistrength© C100 appears of low concern in term of local and systemic genotoxicity. Published in: Pothmann D et al. Lung inflammation and lack of genotoxicity in the comet and micronucleus assays of industrial multiwalled carbon nanotubes Graphistrength © C100 after a 90-day nose-only inhalation exposure of rats. Particle and Fibre Toxicology 2015, 12:21.

3488 The Occupational Life Cycle of MWCNT: Toxicity Evaluation from As-Produced to Post-Production Modifications and Composites

L. M. Bishop[2], M. Orandle[2], L. Cena[2], V. Kodali[2], M. Dahm[1], M. Schubauer-Berigan[1], T. Sager[2], J. Scabilloni[2], D. Schwegler-Berry[2], T. Eye[2], L. Battelli[2], J. Kang[2], G. Casuccio[3], K. Bunker[3], A. Stefaniak[2], P. C. Zeidler-Erdely[2], L. Sargent[2], R. R. Mercer[2] and A. Erdely[2]. *[1]National Institute for Occupational Safety and Health, Cincinnati, OH; [2]National Institute for Occupational Safety and Health, Morgantown, WV and [3]RJ Lee Group, Monroeville, PA.*

Companies synthesize or purchase as-produced multi-walled carbon nanotubes (AP-MW), apply a polymer coating (PC-MW), then embed the PC-MW into an epoxy or fiberglass matrix. Workers are potentially exposed at each production stage from dry powder handling of the MW or aerosols from sanded composites. To date, toxicity studies have focused primarily on AP-MW. The aim was to characterize the toxicity of these additional materials. Materials were prepared to emulate workers personal breathing zone collections and male C57BL/6J mice were then dosed by oropharyngeal aspiration with vehicle, AP-MW, or PC-MW from two separate companies at 4 µg or 40 µg. Mice were sacrificed at 4 h, 1, 7, 28 and 84 d post-exposure with collection of lavage fluid, lung, tracheobronchial lymph node (TBL), kidney, and liver. Both AP-MW induced dose- and time-dependent measures of pulmonary cytotoxicity, inflammatory cell influx, and inflammatory proteins. Histopathology identified small granulomas at terminal bronchioles at 84 d but no significant alveolar fibrosis at the 40 µg dose. In neither case did PC-MW enhance the effects of AP-MW. With mass as a dose metric, inflammation was less from exposure to PC-MW from company #2 and, at 84 d, remaining material was localized to large extracellular aggregates with minimal or no inflammation. All MW were mostly cleared by 84 d. Translocation to the TBL was more prevalent for materials from company #2 but unlike larger diameter MW, no translocation to systemic organs was detected for any material. Lastly, the PC-MW containing composites with 0.15% and 3% PC-MW by weight were subjected to an industrial sanding process. Assessment of generated aerosols show primarily micron size particles with some MW protrusions (evidence of free MW under evaluation). These findings provide toxicity context at different stages of the MW production chain.

3489 Pulmonary Effects of Different Sized and Oxidized Forms of Graphene Nanoparticles

J. A. Thompson, J. S. Fedan, T. Sager and J. R. Roberts. *PPRB, National Insitute for Occupational Safety & Health, Morgantown, WV.*

Risk of graphene (Gr) nanoparticle inhalation by workers during the manufacture of composite materials is a growing concern as new graphene nanomaterials are generated and utilized. Gr-induced cytotoxicity *in vitro* (PC12 cells, fibroblasts) and lung inflammation *in vivo* have been demonstrated, and Roberts et al (2013) showed that lung inflammation was increased up to 7 d after aspiration of non-oxidized Gr particles with lateral dimensions >5 µM and greater number of layers (~20) compared with that of smaller particles (<1 µM, ~4 layers). We reported previously that a single exposure of graphene [5 µm lateral x 7-10 nm thick (Gr5)] transiently increased lung resistance (RL), dynamic lung compliance (CDyn) and reactivity to inhaled methacholine (MCh) in mice. In this study we examined the effects two additional sizes of non-oxidized Gr particles [20 µm lateral x 7-10 nm thick (Gr20), and <2 µm lateral x 1-2 nm thick (Gr1)] as well as a reduced form of oxidized Gr (rGO), on basal RL and CDyn and reactivity to inhaled methacholine (MCh). Mice were given 40 µg Gr (Gr1, Gr20 or rGO) suspended in dispersion medium (DM; control) via aspiration. Basal levels and RL and CDyn responses to increasing concentrations of inhaled MCh were measured 4 h - 2 mo after Gr exposure. Gr1 increased basal RL on day 7 and basal RL decreased at 2 mo. Gr1 increased Cdyn responses to MCh 1 mo post-exposure, which returned to control levels at 2 mo. However, basal Cdyn was increased by 2 mo post-exposure. Gr20 increased Cdyn responses to MCh 1 mo post-exposure, but Cdyn returned to control by 2 mo. rGO had no effect on basal RL or Cdyn, nor MCh reactivity. Our results indicate that exposure to non-oxidized Gr nanoparticles resulted in transient changes in RL or Cdyn responses to MCh, while the reduced form rGO had no significant effect on airway reactivity or basal RL or Cdyn. Basal Cdyn was increased 2 mo after exposure to Gr1. Our findings suggest that the pulmonary effects of graphenes are influenced by particle size, number of layers and oxidative/reduced state.

3490 Identification of Biomarkers for Nano-Safety Assessment through Proteomic Analysis of Multi-Walled Carbon Nanotubes Functionalized by Atomic Layer Deposition Coating

G. M. Hilton, A. Taylor, E. Dandley, G. Parsons, J. Bonner and M. Bereman. *Biological Sciences, North Carolina State University, Raleigh, NC.* Sponsor: J. Bonner.

Introduction: Multi-walled carbon nanotubes (MWCNT) are commonly surface-functionalized post-synthesis to enhance their novel properties for use in electronics and engineering. Since MWCNT are known to cause lung fibrosis in rodents, it is important to determine how functionalization affects pro-fibrotic potential in order to predict and prevent fibrosis. Preventative measures can be acquired through the identification and monitoring of groups of protein biomarkers. The goal of this work was to use proteomics to identify biomarkers of fibrosis unique to MWCNT atomic layer deposition (ALD) coating type. Methods: Mouse epithelial cell culture was established using E10 cells derived from normal type-II epithelial cells. E10 cells were exposed to control media, uncoated (U)-MWCNT and two ALD-coated MWCNTs: aluminum oxide (A), and zinc oxide (Z). Following a 24 hour exposure, cells were harvested, lysed, trypsin digested, and the peptides were isolated. Nanoflow LC-MS/MS was conducted using a ThermoScientific Q-Exactive Plus, and further validation of significant proteins was achieved using a triple quadrupole mass spectrometer, Quantiva. Spectra were searched using Sequest implemented through Proteome Discoverer. Skyline-daily was used to evaluate significant proteins of interest by selected reaction monitoring. Results: The E10 exposure yielded several differentially expressed proteins across coating types that perturbed pathways by different mechanisms; including: disruption in oxidative phosphorylation, and complement activation. A two-sample t-test found the following number of significant proteins for each exposure group compared to control (α=0.05): 167 Z-MWCNT (2.5 µg/mL), 199 A-MWCNT (100 µg/mL), and 142 U-MWCNT (100 µg/mL). Conclusion: *In vitro* proteomic experimentation was able to elucidate differential pathway disruption as a function of MWCNT coating type. Funding: Supported by NIEHS grants T32 ES007046 and R01ES020897.

3491 Toxicity of Carbon Nanotubes *In Vivo*: Biochemical and Histopathological Parameters

J. J. Guzmán-Mendoza[4], B. E. Sánchez-Ramírez[4], S. L. Montes-Fonseca[1], M. d. C. González-Horta[4] and E. Orrantia-Borunda[2]. *[1]Tecnologico de Monterrey Campus Chihuahua, Chihuahua, Chih., Mexico; [2]CIMAV, Centro de Investigación y Estudios Avanzados, Chihuahua, Chih., Mexico; [3]Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua, Chihuahua, Chih., Mexico and [4]Laboratorio de Biotecnología III, Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua, Chihuahua, Chih., Mexico.* Sponsor: L. Mía. del Razo Jiménez.

Carbon nanotubes (CNT) are cylindrical nanoparticles with biomedical applications due to their ability to be functionalized with chemical substances. However, toxicity in *in vivo* models is not yet fully established and the results are contradictory. The objective of this project was to evaluate the toxic effect of different NTC *in vivo* model using biochemical and histopathological parameters. For this purpose, CNTs were synthesized by spray pyrolysis (UP-CNTs) and purified (P-CNTs) using sonication for 48 h. The P-CNTs were functionalized with fluorescein isothiocyanate (FITC-CNTs). Balb/c mice were exposed to a single intravenous dose of 60 µg/mouse: NP-NTC, P-NTC, FITC-NTC; or 0.2% pluronic F-127 as vehicle control. The toxicity testing was performed by determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea and creatinine metabolites in venous blood samples before exposure to the different CNTs, and 24 h, 14, 29 and 60 days post-exposure. The histopathological damage was assessed by dissecting and histological analysis of sections from liver, lung, kidney and spleen. The results showed high toxicity in animals exposed to UP-CNTs compared with other CNTs, showing extensive lesions in the lungs, kidneys and liver. Biochemical parameters in this group had a significant increase, related to hepatotoxicity and kidney damage. In the case of animals exposed to FITC-CNTs, these revealed toxic effects to a lesser degree, it was attributed to FITC. However, mice exposed to the P-NTC showed no significant minimal differences compared to control. These results suggest that the purification is a determining factor *in vivo* toxicity of CNTs, postulating the P-CNTs as potential candidates for biomedical applications.

3492 Multi-Walled Carbon Nanotube (MWCNT)-Induced Fibrogenic Signaling in a Human Lung Epithelial-Fibroblast Co-Culture System

A. L. Mihalchik[1,2], J. D. Sisler[1] and Y. Qian[1]. *[1]NIOSH, Morgantown, WV and [2]West Virginia University, Morgantown, WV.*

It has been established that MWCNT induce pulmonary fibrosis in *in vivo* models. *In vitro* studies of fibroblasts in monoculture have also investigated MWCNT toxicity. However, the role of other cell types and cellular communication in the mechanisms of MWCNT-induced fibrosis needs further study. Our lab previously elucidated inflammatory and vascular effects of MWCNT exposure in an epithelial-endothelial co-culture model; here, we employ a system to examine fibrogenic responses to MWCNT. In this study, we assessed the effects of pristine MWCNT at an occupationally relevant dose on fibroblasts grown in a transwell co-culture system of apical Beas-2B human bronchial epithelial cells and basolateral WI-38 normal human lung fibroblasts. To address the impact of cell-to-cell communication between the epithelium and underlying fibroblasts on pulmonary fibrosis, we first measured chemokine (C-C motif) ligand 2 (CCL2) concentrations by ELISA in monoculture and co-culture conditioned media. CCL2 levels were significantly elevated in the basolateral chamber of MWCNT-exposed cells in co-culture, without similar changes in WI-38 monoculture or unexposed WI-38 grown in the co-culture system. CCL2 has been shown to be increased and upregulated in patients with idiopathic pulmonary fibrosis, usual interstitial pneumonia, and bronchiolitis obliterans syndrome, and responsible for fibroblast production of TGF-β and procollagen. In further support, MWCNT-exposed WI-38 in co-culture exhibit increased collagen type I, a hallmark of fibrosis, as compared to unexposed WI-38 in co-culture. These results suggest that the epithelial-fibroblast co-culture system may be more reflective of the fibrotic disease state instead of monoculture systems, and may allow us to further elucidate the mechanism of MWCNT-induced fibrosis *in vitro*.

3493 Thirteen-Week Nose-Only Inhalation Study in Rats of Graphistrength© C100 Multiwalled Carbon Nanotubes (MWCNT) with 13- and 52-Week Recovery Periods

J.-F. Régnier[4], D. Pothmann[2], J.-L. Le Net[3] and J. Beausoleil[1]. *[1]Arkema France, Lacq, France; [2]Harlan Laboratories Ltd, Itingen, Switzerland; [3]Le Net Pathology Consulting, Amboise, France and [4]Toxicology and Environment, Arkema France, Colombes, France.*

Graphistrength© C100 provides superior electrical and mechanical properties for various applications. Graphistrength© C100 is formed of MWCNT (ca. 12 walls, outer mean diameter ca. 12 nm, length ca. 1 µm) agglomerated in particles with a granulometry centered on 400 µm. After investigating different parameters for the aerosol generation, male and female Wistar rats were exposed by nose-only inhalation (6h/day, 5d/week) to target concentrations of 0.05, 0.25 and 5.0 mg/m3 air of a respirable aerosol (MMAD < 3 µm) and sacrificed 24 hours after 4 weeks and 24 hours, 13 and 52 weeks after 13 weeks of exposure. Clinical, biological and histological evaluations were performed according to the OECD TG 413. Broncho-alveolar lavage fluid (BALF) was also collected and analyzed for cytokines and inflammatory parameters. Deposition of black particles (MWCNT) in lungs was observed at all test concentrations. An inflammatory lung reaction was observed in all rats exposed to 5.0 mg/m3. It was characterized by changes in the cytological, biochemical and cytokine parameters of BALF, an increase in the neutrophil blood cells count, an increase of the lung weight, an interstitial inflammation mainly around the alveolar ducts at the bronchiole-alveolar junction and a cell hypertrophy/hyperplasia in the terminal and respiratory bronchioles. The slight changes in BALF parameters observed at 0.25 mg/m3 recovered after a 13-week treatment-free period. Signs of lung clearance of the MWCNT were observed at 0.05 and 0.25 mg/m3. No pathological changes were observed on the pleura nor was there any brain translocation via the olfactory bulb. In conclusion, a lung inflammation characteristic of overload with insoluble particles was observed after a 90-day exposure to 5.0 mg/m3 of Graphistrength© C100, a No-Observed Adverse Effect Concentration (NOAEC) of 0.25 mg/m3 was established for the repeated-dose toxicity. Published in: Pothmann D et al. Lung inflammation and lack of genotoxicity in the comet and micronucleus assays of industrial multiwalled carbon nanotubes Graphistrength © C100 after a 90-day nose-only inhalation exposure of rats. Particle and Fibre Toxicology 2015, 12:21.

3494 Acute Phase Inflammatory Responses in Mice after Pulmonary Exposure to Carbon Nanotubes Functionalized by Zinc Oxide Thin Film Coating

E. C. Dandley, A. J. Taylor, K. S. Duke, K. A. Shipkowski, G. N. Parsons and J. C. Bonner. *Department of Biological Sciences, North Carolina State University, Raleigh, NC; Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.*

Introduction. Multi-walled carbon nanotubes (MWCNT) are used for a wide range of applications in material fabrication, electronics and medicine. Despite these benefits, MWCNTs cause lung inflammation and fibrosis in mice after inhalation exposure, suggesting a possible human health hazard. Atomic layer deposition (ALD) is a method for applying conformal nanoscale coatings to enhance/alter surface properties. We previously reported that ALD coating of MWCNTs with Al2O3 reduced cytokines in human monocytes *in vitro* and decreased lung fibrosis in mice compared to uncoated MWCNTs. Here, we investigated the lung inflammatory and fibrogenic responses of monocytes (THP-1 cells) and mice to MWCNTs coated by ALD with ZnO (Z-MWCNTs). Methods. MWCNTs were coated with ZnO to yield Z-MWCNTs. THP-1 cells were assayed for cytokine mRNA levels after treatment with uncoated MWCNT or Z-MWCNTs. Mice were exposed to MWCNT or Z-MWCNTs by oropharyngeal aspiration (4 mg/kg) and evaluated for lung inflammatory cell counts, histopathology and cytokines at day 1 and 28 post-exposure. Results. Z-MWCNTs or ZnO nanoparticles enhanced IL-6, IL-1β, CXCL10 and TNF-α mRNA levels in THP-1 cells *in vitro* relative to MWCNTs at 1 day. Mice exposed to Z-MWCNT had increased lung inflammation and bronchoalveolar lavage fluid (BALF) cells. IL-6, CXCL10 and TNF-α mRNAs were increased in lung tissue by Z-MWCNTs at 1 day. However, Z-MWCNTs increased only BALF protein levels of IL-6 and CXCL10. IL-6 mRNA was increased in the heart, liver and spleen of Z-MWCNT-treated mice. Lung fibrosis was not different between mice treated with uncoated or Z-MWCNTs 28 days post exposure. Conclusions. These findings indicate that ALD coating of MWCNTs with ZnO enhances acute lung inflammation in mice and therefore, induction of IL-6 in THP-1 cells *in vitro* predicts lung and systemic responses to Z-MWCNTs *in vivo*.
Funding: Supported by NIEHS grant R01-ES020897

3495 Utilization of Near Infrared Fluorescence Imaging to Track and Quantify Pulmonary Retention of Single-Walled Carbon Nanotubes in Mice

J. N. Nicholas[3], H. Chen[3], J. Besisi[3], P. L. Ferguson[1], K. Lui[1], D. Bolser[3] and T. Sabo-Attwood[3]. *[1]Civil and Environmental Engineering, Duke University, Durham, NC; [2]Environmental and Global Health, University of Florida, Gainesville, FL and [3]Physiological Sciences, University of Florida, Gainesville, FL.*

Adequate detection techniques to examine the biotoxicity from pulmonary exposures of single-walled carbon nanotubes (SWNTs) present a unique challenge. Traditional analytical methods that effectively assess their distribution *in vivo* lack spatial resolution for differentiating carbon nanomaterials from the carbon in the biomass of living cells. Near infrared fluorescence (NIRF) imaging has emerged as a valuable tool for utility in studies for detection and quantification of single-walled carbon nanotubes (SWNTs) *in vivo*. The objective of this study was to develop and optimize NIRF-based imaging and quantitation methods for tracking and quantifying SWNTs in a murine pulmonary exposure model. We designed a custom NIRF system that allows for visualization, semi-quantitation and spectroscopic analysis of SWNTs in whole organs and histological tissue sections. To assess biopersistence *in vivo*, C57BL/6 mice were challenged with a single dose of SWNTs by pharyngeal aspiration (20 ug/mouse), and their distribution was tracked using NIRF imaging at 3, 7 and 14 days. Spectroscopic analysis revealed 90% recovery of SWNTs from normal lung tissues. Semi-quantitative analysis of images showed strong and innate SWNT-derived fluorescence throughout whole lungs tissues at all-time points measured. This was consistent with data from spectroscopic quantitation analysis; overall results showed a steady level of SWNT at each time-point with minimal clearance observed. Additionally, histopathological examination of airway perfused lungs revealed that SWNTs were present in the terminal bronchioles, alveolar ducts and proximal acinar alveoli and associated with inflammatory cell infiltrates dominated by macrophages in bronchiolar and alveolar spaces. Adverse impacts on viability and health condition of exposed mice were not observed during the study. Results of this study show pulmonary retention of SWNTs up to day 14 post initial exposure and highlight the utility of NIRF as a tool to assess clearance and distribution of SWNTs *in vivo*. This work is imperative as processes of clearance are vital in understanding long-term health impacts observed in other particulates.

3496 Characterization of Lung Toxicity Following Pulmonary Exposure to Graphene Nanoparticles in Different Oxidized Forms

J. R. Roberts[2,3], T. Sager[2,3], L. Bishop[2,3], R. R. Mercer[2], A. B. Stefaniak[2], N. V. Yanamala[2], S. S. Leonard[2,3], K. A. Roach[2,3], D. Schwegler-Berry[2], I. S. Chaudhuri[1], A. Kyrlidis[1], B. Y. Farris[2,3], C. E. McLoughlin[2], T. Eye[2], V. Kodali[2] , M. Wolfarth[2], D. W. Porter[2,3], V. Castranova[3] and A. Erdely[2,3]. *[1]Cabot Corporation, Billerica, MA; [2]CDC/NIOSH, Morgantown, WV and [3]West Virginia University, Morgantown, WV.*

As the manufacturing of graphene nanoparticles (GNP) increases, there is concern for the health effects that due to lung exposure in workers. This study evaluated lung toxicity of different oxidized forms of GNP: non-oxidized graphite nanoplates (Gr), an oxidized intermediate (GO), and the reduced form of GO (rGO), with multi-walled carbon nanotubes (MWCNT, Mitsui-7) as a particle control. Male C57BL/6J mice received 4 or 40 µg of Gr, GO, rGO, MWCNT, or dispersion medium (DM) by oropharyngeal aspiration. Bronchoalveolar lavage (BAL) was performed at 4 hr, 1 d, 7 d, 1 m, and 2 m post-exposure. In separate mice, lungs were preserved for pathology and RNA analysis. Lung injury was elevated in MWCNT and rGO at 40 µg when compared to all groups at all times, with the greatest increase in the MWCNT group at 1 and 7 d, and in the rGO group at 1 and 2 m. Gr and GO at 40 µg, and MWCNT at 4 µg increased injury at early time points only. Alveolar macrophages (AM) in BAL were elevated in all 40 µg groups at 7 d, with the greatest increase in the rGO animals at 7 d and 1 m. This response persisted for the rGO and MWCNT 40 µg groups up to 2 m. BAL neutrophils were increased in all 40 µg groups up to 7 d, and persisted in the MWCNT and rGO groups up to 2 m. 4 µg doses of rGO and MWCNT also caused increased neutrophils at early time points. Acutely, all 40 µg exposures increased relative mRNA expression of Cxcl2, Ccl2, Il6, and Ccl22. The effects persisted 1 m in the Gr and GO 40 µg dose and MWCNT 4 µg dose, and 2 m for rGO and MWCNT 40 µg groups. Histopathology showed fibrosis at 7 d in MWCNT and rGO 40 µg groups scored as minimal, which persisted throughout the time course, and mild type II epithelial hyperplasia and inflammation, which resolved over time. Gr and GO caused transient inflammation/injury, whereas rGO caused comparable toxicity to MWCNT. Differences in toxicity may be due to chemistry, shape, and/or density of materials, with a higher volume load per mass delivered for the low density particles, MWCNT and rGO.

3497 Atomic Layer Deposition Coating of Multi-Walled Carbon Nanotubes with Metal Oxides Alters Pro-Fibrogenic Responses in an *In Vitro* Tri-Culture System of the Pulmonary Alveolus

A. J. Taylor, E. C. Dandley, G. N. Parsons and J. C. Bonner. *Department of Biological Sciences, North Carolina State University, Raleigh, NC; Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.*

Introduction. Multi-walled carbon nanotube (MWCNT) inhalation exposure is a potential risk to human health due to their increasing use in consumer products. Atomic layer deposition (ALD) is a method for applying conformal nanoscale coatings to enhance surface properties of materials. In rodent models, pulmonary exposure to MWCNTs causes increased inflammation & fibrosis. Recently, we have determined that ALD coating of MWCNTs with aluminum oxide decreased fibrosis and pro-fibrogenic cytokines (OPN & TGF-β1) induced by uncoated MWCNTs in lungs of mice. The purpose of this study was to determine whether ALD coating with metal oxides such as aluminum (A), zinc (Z) or titanium oxide (T) would alter the fibrogenic response to MWCNTs. Methods. Uncoated (U-) MWCNTs or ALD-coated MWCNTs (A-, Z-, T-MW) were incubated with THP-1 macrophages, BEAS-2B epithelial cells, & HLF-19Lu fibroblasts alone or in co-/tri-culture. Pro-fibrogenic mediator analysis included: TGF-β1, OPN, and collagen mRNAs (Col1a1 & Col1a2). qRT-PCR was performed to assess mRNA expression. Results. With THP-1s alone, all MWCNTs increased Col1a1 mRNA at 48 & 72hr but only Z-MW increased Col1a2, OPN, & TGF-β1 at 72hr. U-MW induced Col1a1 & Col1a2 mRNA at 24hr at the highest dose (50 & 100µg/ml). High doses of Z-MW (10, 50, 100µg/ml) increased Col1a2 substantially but decreased OPN & TGF-β1 mRNAs. In BEAS-2Bs, U-MW, A-MW and Z-MW exposure increased TGF-β1 mRNA with U-MW having the most effect. Changes in mRNA seen in cells cultured alone were dampened when in co-culture with the exception of OPN where mRNA levels were increased when THP-1s were in co-culture with HLFs. In the tri-culture model, Z-MW increased Col1a1 & Col1a2 mRNA at 24hr. A-MW & T-MW had no effect on Col1a1 & Col1a2 in tri-culture. Conclusions. hese findings indicate that ALD coating of MWCNTs alters pro-fibrotic mediators *in vitro* with Z-MW increasing fibrogenic activity and A- or T-MW decreasing fibrogenic activity. Funding: Supported by NIEHS grant R01-ES020897

3498 Hyaluronan Functionalization Reduces Lung Inflammatory and Fibrotic Responses of Multi-Walled Carbon Nanotubes

S. Hussain[3], Z. Ji[1], A. Taylor[4], L. Miller-DeGraff[3], M. George[3], J. Marshburn[3], R. Snyder[3], A. Rice[3], J. C. Bonner[4] and S. Garantziotis[3]. *[1]UCLA, Los Angeles, CA; [2]Clinical Research Program, NIEHS/NIH, Research Triangle Park, NC; [3]IIDL, NIEHS/NIH, Research Triangle Park, NC and [4]Toxicology Program, NC State University, Raleigh, NC.*

Multi-walled carbon nanotubes (MWCNT) have promising applications in the fields of nanotechnology, nano diagnostics and nanomedicine. However, there are possibilities of human health risk especially for lung diseases as a result of inhalation exposures. Hyaluronic acid/hyaluronan (HA), a high molecular weight oligosaccharide, is one the major components of lung extracellular matrix. The high molecular weight isoform of HA has anti-inflammatory properties. We hypothesized that grafting high molecular weight HA on MWCNTs can reduce their toxicity. We further aimed at comparing the toxicity of commercially available (as prepared), purified and functionalized nanotubes with hyaluronan grafted MWCNT (MWCNT-HA). Nanotube were characterized by transmission electron microscopy, FTIR, ICP-OES and Raman spectroscopy while their suspensions were analyzed using Dynamic Light Scattering. Toxicity of nanotubes was evaluated in human lung fibroblasts and in mouse lungs (after oropharyngeal aspiration of 1.5mg/Kg nanotubes). Mice were sacrificed either at day 1 or day 21 after nanotube exposure; lungs were lavaged and collected for histology and protein/RNA isolations. MWCNT-HA induce lower cellularity, TNF-alpha production and HA levels in BAL of mice than all other MWCNT preparations. Histological scoring further indicated reduced perivascular and particle-associated inflammation, collagen deposition and epithelial proliferation in MWCNT-HA treated mouse lungs compared to other MWCNT preparations. In human lung fibroblasts, MWCNT-HA exposure results in reduced pro-fibrotic gene expression however it induces proliferation.

Taken together we demonstrate that HA grafting results in reduced lung inflammation by MWCNT in rodents. However, it induces proliferation of human lung fibroblasts. Further research is currently being conducted to understand the molecular mechanisms involved in MWNCT-HA induced cellular protection.

3499 No Immunohistologically Detectable Genotoxicity or Proliferation Following 90day Inhalation of Multi-Walled Carbon Nanotubes

D. Schaudien[1], A. Hackbarth[1], H. Ernst[1], S. Rittinghausen[1], A. Leonhardt[2], U. Heinrich[1] and O. Creutzenberg[1]. [1]*Fraunhofer ITEM, Hannover, Germany and* [2]*Leibniz Institute for Solid State and Materials Research, Dresden, Germany*. Sponsor: C. Dasenbrock.

Introduction: Nanomaterials are gaining more importance in science and medicine. However, recently concerns about possible carcinogenicity of carbon nanotubes have been raised. Methods: To investigate genotoxicity and proliferation caused by carbon nanotubes, 3 different multi-walled carbon nanotubes varying in length and diameter which previously showed carcinogenic potency after intraperitoneal injection (Rittinghausen et al., PartFibreToxicol 2014) were used in a 90-day nose-only inhalation study in Wistar rats. Amosite asbestos served as fiber, carbon black (CB) as particle, and clean air as negative control. The concentration of aerosol was 100 WHO fibers/ml. CB concentration was 5mg/m3. After 16 and 94 recovery days, the lung was processed for histopathology and immunohistology using antibodies to detect 8-OH-dG, y-H2AX, and cell proliferation(BrdU). Results: Histologically, rats which inhaled asbestos or carbon black exhibited more severe changes compared to those inhaled multi-walled carbon nanotubes. Immunohistological examination of genotoxicity using antibodies against 8-OH-dG and y-H2AX revealed no increase of positive cells in any group. Cell proliferation was increased in lung parenchyma of rats which inhaled asbestos or CB. Furthermore, rats exposed to asbestos showed an increase of cell proliferation at the terminal bronchioli but no increase was detectable in lung pleura. Multi-walled carbon nanotubes did not lead to an increase of cell proliferation in any lung compartment investigated. Discussion: Interestingly, inhalation of multi-walled carbon nanotubes which showed a carcinogenic potency did not result in an increase in 8-OH-dG- or y-H2AX-positive cells nor cell proliferation. Probably, this is due to the much lower fiber dose in lungs as compared to that used in the i.p. test.

3500 Adjuvant Effects of Multi-Walled Carbon Nanotube Sensitization with House Dust Mite Allergen Lead to an Exaggerated Asthmatic Phenotype in Mice

M. D. Ihrie, K. S. Duke, A. J. Taylor and J. C. Bonner. *Department of Biological Sciences, North Carolina State University, Raleigh, NC.*

Introduction: Multi-walled carbon nanotubes (MWCNTs) have numerous applications in electronics, engineering and medicine. There is increasing evidence that MWCNTs can have harmful effects upon inhalation. Of particular susceptibility are individuals with asthma, an allergic lung disease characterized by a TH2 immune response, airway hypersensitivity, mucus cell metaplasia and airway remodeling. We hypothesized that repeated intranasal (IN) exposure to either tangled (t) or rigid (r) MWCNTs would sensitize the immune system to cause an asthma-like phenotype upon subsequent challenge with MWCNTs. Methods: Male C57BL6 mice were dosed via IN aspiration on days 0, 2, 4, 14, 16 and 18 with either vehicle, HDM allergen (25 µg), t or rMWCNTs (0.5 mg/kg), or a combination of HDM and each MWCNT; n=39. Necropsy was performed on days 21-22 and bronchoalveolar lavage fluid (BALF), serum and organs were collected. Results: BALF from HDM/MWCNT treated mice showed elevated leukocyte influx, with HDM treatment causing eosinophilia which was enhanced by MWCNT co-exposure. MWCNTs were observed in alveolar macrophages, although only a small fraction of tubes delivered by IN reached the lungs. Serum IgE was significantly enhanced in the rMWCNT/HDM group, but not the tMWCNT/HDM group, over vehicle and HDM. BALF levels of TGF-β1, OPN, IL-13 and IL-1β were not altered, while CCL2 was increased by HDM/tMWCNT treatment. Lung histology showed little effect of MWCNT treatment alone, while HDM caused lymphocyte influx, airway wall thickening and enhanced mucus production. These lung inflammatory effects were greatly enhanced by HDM/MWCNT co-exposure. Conclusions: Sensitization of mice with t or rMWCNT caused a strong adjuvant-like effect with HDM to promote an allergic inflammatory phenotype. These findings suggest that either t or rMWCNT represent a health risk to individuals with allergic airway disease. Funding: Supported by NIEHS grant R01-ES020897.

3501 Epigenetic Alterations in Response to the Inhalation of Different Sized Multi-Walled Carbon Nanotubes

J. Ray[1], V. Kay[2], J. W. Lee[2], E. Cole[2], A. Holian[2] and Y. H. Cho[2]. [1]*Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI and* [2]*The Center for Environmental Health Sciences, University of Montana, Missoula, MT.*

Multi-walled carbon nanotubes (MWCNT) have been shown to cause significant pathological changes in animal models, particularly in the airways. This raises concern that adverse human health effects will emerge with increasing use and exposure of these materials. We have reported that the bioactivity of MWCNT increases with size (diameter & length). However, the mechanism for MWCNT bioactivity has not been extensively studied. Epigenetic changes represent a mechanism for the transcriptional regulation of gene activity and numerous studies address the role of epigenetic regulation following environmental exposures and impact on inflammation. Here, we investigate epigenetic biomarkers for exposure to different sizes of MWCNT and their relationship to MWCNT-induced pulmonary fibrosis and inflammation. Oropharyngeal aspiration was used to expose C57BL/6 mice to 50µg of different sizes of MWCNT (narrow short (NS), wide short (WS), and narrow long (NL)). Whole lung tissue was collected at 1 and 7-days post exposure for DNA methylation analysis. Global DNA methylation and gene-specific methylations of IFN-γ, IL-6, CXCL1, and Thy-1 were measured using luminometric methylation and pyrosequencing assays, respectively. The results showed significant global hypomethylation 1-day post exposure to the NL particles. On day 7 following exposure to the NL particles, CXCL1 was significantly hypomethylated. Additionally, IL-6 methylation was significantly decreased 1-day post exposure to the NS and (borderline) NL particles. This data suggests that different sized MWNCT cause differing methylation patterns in selected genes, and exposure to NL particles induces significant epigenetic alterations. This corresponds with previous data showing that the biological activity of these particles follows the pattern NL > WS > NS. Our results support the idea that nanoparticle toxicity cannot be classified solely by elemental composition, but must also take size into account.

3502 Susceptibility of STAT1-Deficient Mice to Pulmonary Fibrosis after Exposure to Tangled vs. Rigid Multi-Walled Carbon Nanotubes

K. S. Duke, E. A. Thompson, K. A. Shipkowski, M. D. Ihrie, A. J. Taylor and J. C. Bonner. *Department of Biological Sciences, North Carolina State University, Raleigh, NC.*

Introduction: Multi-walled carbon nanotubes (MWCNT) are a potential risk for pulmonary fibrosis due to their fiber-like shape, but other physico-chemical features such as rigidity could also confer fibrogenicity. The signal transducer and activator of transcription-1 (STAT1) is an important anti-fibrogenic transcription factor that promotes fibroblast growth arrest and STAT1 deficient (STAT1-/-) mice are susceptible to pulmonary fibrosis. In this study, we hypothesized that STAT1-/- mice or primary STAT1-/- lung fibroblasts exhibit a differential fibrogenic response to tangled (t-) vs. rigid (r-) MWCNT above that seen with wild-type (STAT1+/+) mice or STAT1+/+ lung fibroblasts. Methods: Primary mouse lung fibroblasts (MLF) were isolated from STAT1+/+ or STAT1-/- mice, exposed to a t- or rMWCNT (10 µg/ml) and analyzed for protein expression of fibrogenic mediators by Western blot. STAT1+/+ and STAT1-/- mice were exposed to t- or rMWCNT (4 mg/kg) via oropharyngeal aspiration and lung tissues were collected after one and 21 days to measure mRNA and protein levels of fibrogenic mediators. Results: rMWCNT caused greater fibrosis and more granulomas than tMWCNT in WT mice and this was further enhanced in STAT-1-/- mice. rMWCNT induced eosinophilia while tMWCNT induced neutrophilia. STAT1-/- mice treated with rMWCNT had higher TGF-β1 protein levels in bronchoalveolar lavage fluid after 21 days than STAT1+/+ mice. p53 mRNA levels were elevated in the lungs of STAT1-/- mice compared to STAT1+/+ after t- or rMWCNT exposure. STAT1-/- MLF had increased p53 expression compared to STAT1+/+ MLF; p53 protein levels were further increased by rMWCNT > tMWCNT. Conclusions: r- and tMWCNT induce different pulmonary fibrogenic responses that are exaggerated by STAT1 deficiency, emphasizing the importance of tube rigidity and genetic susceptibility. The mechanism of STAT1 susceptibilty to MWCNT-induced fibrosis appears to be through dysregulated TGF-β1 and p53 expression. Funding: Supported by NIEHS grant R01-ES020897.

3503 Pulmonary Exposure to Multi-Walled Carbon Nanotubes Causes Unique Immunotoxic mRNA Profiles in the Lungs, Liver, and Spleen of Mice Sensitized to House Dust Mite Allergen

K. A. Shipkowski[1], A. J. Taylor[1], E. A. Thompson[1], E. E. Glista-Baker[1], B. C. Sayers[1], K. S. Duke[1], J. H. Shannahan[2], J. M. Brown[2] and J. C. Bonner[1]. *[1]Department of Biological Sciences, North Carolina State University, Raleigh, NC and [2]Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO.*

Pulmonary exposure to multi-walled carbon nanotubes (MWCNTs) exacerbates airway inflammation in animal models of pre-existing allergic asthma, but little research exists on the extra-pulmonary effects of MWCNTs, particularly following allergen sensitization. T helper (Th) immune responses to MWCNT exposure during allergic airway disease are regulated by specific transcription factors and downstream cytokine signaling. The goal of this study was to better understand the systemic effects of MWCNT exposure in mice previously sensitized with house dust mite (HDM) allergen through an immunological survey of specific factors involved in Th immune responses. C57BL6 mice were sensitized with HDM allergen, exposed to MWCNTs, and evaluated 21 days post-MWCNT exposure. Taqman qRT-PCR was utilized to measure mRNA expression of Th-related transcription factors and cytokines in the lungs, liver, and spleen of exposed animals. HDM sensitization and MWCNT exposure caused enhanced airway remodeling in the lungs of mice and induced distinct immunological profiles in the lungs, liver, and spleen of mice. STAT3 mRNA expression was significantly increased in the lungs, liver, and spleen of mice exposed to HDM/MWCNTs. In contrast, levels of STAT1 mRNA and downstream signaling molecules IFN-γ and CXCL10 were increased specifically in the liver of mice sensitized with HDM alone. HDM/MWCNT exposure increased FoxP3 and TGF-β1 mRNA in the lungs and IL-10 mRNA in the lungs and spleen. HDM/MWCNT treatment also enhanced lung mRNA expression of IL-13 and GATA3, both Th2-related transcription factors. These data indicate that exposure to MWCNTs following HDM sensitization can alter Th transcriptional responses in the lungs, liver, and spleen of exposed animals. These tissue-dependent responses suggest the potential for toxic extra-pulmonary effects following MWCNT exposure and increased disease susceptibility during allergic airway inflammation. Funding: Supported by US Public Health Service Grants NIEHS R01 ES020897 (JCB), R01 ES019311 (JMB), and K99 ES024392 (JHS).

3504 Metallic-Doped Carbon Nanotubes Show Minimal Toxicity to Nasal Epithelial Cells

M. W. Grogg[2], V. Gogineni[2], L. K. Braydich-Stolle[2], S. Ganguli[2], A. K. Roy[2] and S. M. Hussain[2]. *[1]RHDJ, Air Force Research Laboratory, WPAFB, OH and [2]RXAN, Air Force Research Laboratory, WPAFB, OH.*

Carbon nanotubes (CNTs) have been incorporated into a wide range of consumer products, and reusable sorbent scaffolds to remove oil contamination have been developed from metallic-doped carbon nanotubes. Pulmonary toxicity of multi-walled carbon nanotubes has been extensively studied with reports providing data on cytotoxicity, inflammation, and fibrosis results. In this study, the toxicity of carbon nanotubes doped with varying metals was evaluated in nasal epithelial cells since these are the first respiratory system cells to come in contact with a particular chemical/material during inhalation exposure. According to NIOSH exposure assessment limits, the average daily deposited carbon nanotube dose for humans is ~4 µg, which correlates to an estimated average *in vitro* dose of 64 ng/ml. We investigated cell viability, oxidative stress, cell morphology, and gene expression changes bounding this concentration ranging from 0-1250 ng/ml. The metallic-doped CNTs elicited no observable toxicity in these measures across the entire concentration range over 24 hours. In fact, no significant toxicity was found until a 48 hour exposure at 1250 ng/ml, roughly 20 times the NIOSH daily exposure limit. A similar trend was observed for oxidative stress and cell morphology. When inflammation was evaluated using IL-6 and TNF-α expression, a significant increase was observed at the gene and protein level for both markers at higher concentrations. Taken together, these results indicate that while the metallic-doped CNTs displayed minimal toxicity, there was some minor inflammation generated from exposure at the highest dose and at extended exposure times.

3505 Multi-Walled Carbon Nanotubes May Impair Mucociliary Differentiation of Human Bronchial Epithelia and Club Cell Recovery in Oropharyngeally-Exposed Mice

R. J. Snyder[1,2], S. Hussain[1], A. Rice[1], L. Degraff-Miller[1], S. H. Randell[2] and S. Garantziotis[1]. *[1]National Institute of Environmental Health Sciences, Durham, NC and [2]University of North Carolina Chapel Hill, Chapel Hill, NC.*

Multi-walled carbon nanotubes (MWCNTs) are particularly useful engineered nanomaterials due to their tensile strength, hydrophobicity, and other properties, yet pose a potential inhalation risk for consumers and workers. Previous studies have shown a loss of barrier function, cytoskeletal disruption, and altered retinoid signaling genes in airway epithelial cells exposed to non-cytotoxic doses of MWCNTs. This study investigates whether MWCNTs can impair the regeneration of differentiated epithelial tissues following injury in a mouse model, as well as analyzing markers of mucociliary differentiation in primary human bronchial cells (BECs) exposed to MWCNTs in an air-liquid interface (ALI). BECs from bronchoscopy of healthy patients were grown to confluence on Millicell inserts and were then treated with either dispersion vehicle, nano-graphitized carbon (NG) shape control (4ug/cm2), or MWCNT (0.2, 1, or 4ug/cm2), converted to ALI, and allowed to differentiate for 4 weeks. For *in vivo* studies, 8-week C57Bl/6 female mice were given 200mg/kg naphthalene or corn oil intraperitoneally. After a 24hr recovery, mice were then given 10ug/kg MWCNT or vehicle alone oropharyngeally (equivalent to ~8hr inhaled occupational dose). Mice were sacrificed at days 3, 7, 10, 14, and 21 following dosing. Results *in vitro* demonstrate dose-dependent reduction in ciliated cells by day 28, corresponding to reduced expression of mucociliary differentiation markers Muc5B, FOXJ1, RARRES1, and RDH12. *In vivo*, naphthalene-injured mice treated with MWCNTs recovered less club cell epithelium than injured mice treated with nanographite or vehicle by days 7-14, as measured by immunohistochemical staining for CC-10 and BrdU, as well as transcription of club cell markers CC-10 and CYP2F2. We conclude that MWCNTs impair differentiation of basal bronchial epithelium, prolonging wound repair even at non-toxic doses.

3506 Development of an *In Vitro* Test to Assess the Inhalation Toxicity of Nanomaterials

M. Sharma. *PETA International Science Consortium Ltd., London, United Kingdom.*

The increasing use of multi-walled carbon nanotubes (MWCNTs) in consumer products and their potential to induce adverse lung effects following inhalation has lead to much interest in better understanding the hazard associated with these nanomaterials (NMs). While the current regulatory requirement for substances of concern in many jurisdictions is a 90-day rodent inhalation test, the monetary, ethical, and scientific concerns associated with this assay led an international expert group to convene in February 2015 to discuss the approaches to evaluate inhalation toxicity of MWCNTs. Pulmonary fibrosis was identified as a key adverse outcome linked to MWCNT exposure and recommendations were made on the design of an *in vitro* assay that is predictive of the fibrotic potential of MWCNTs. Subsequently, work began to develop an *in vitro* co-culture system using relevant lung cells exposed to MWCNTs at the air-liquid interface (ALI) while considering human-relevant dosimetry and NM lifecycle transformations. Mono- and co-cultures of human cell lines, including alveolar epithelial cells, fibroblasts, and macrophages were exposed in suspension to MWCNTs (Mitsui 7s dispersed in water with 1% bovine serum albumin) at 3 concentrations (5, 10, and 20 µg/ml) to assess the pro-fibrotic response. No cytotoxicity or increase in inflammatory cytokines was observed 24 hours or 5 days following exposure to monocultures of alveolar cells line (A549). However, this work is complemented by comparative studies using co-culture models and a reconstructed primary human alveolar tissue model (EpiAlveolarTM, MatTek Corp) exposed to NMs at ALI using the VITROCELL Cloud system. The long term goal of this work is to develop an *in vitro* testing strategy using human-relevant methods to predict pulmonary toxicity and to enable effective risk assessment of substances including MWCNTs.

3507 Inhaled MWCNT at Occupational Levels Reduce Alveolar Macrophage Phagocytosis and Vascular Relaxation

D. M. Walters[2], C. D. Worthington[2], M. J. Yaeger[2], J. S. Morgan[2], J. S. Bonner[1], K. M. Gowdy[2] and C. J. Wingard[2]. [1]*Biological Sciences, North Carolina State University, Raleigh, NC and* [2]*Pharmacology and Toxicology, East Carolina University, Greenville, NC;* [3]*Physiology, East Carolina University, Greenville, NC.*

Background: Occupational exposure to inhaled MWCNT are a health concern due to physicochemical properties. While toxicity has been demonstrated, many studies have used high doses and non-physiological exposure routes. The objective of this study was to examine pulmonary and vascular outcomes after inhalation exposure to occupational levels of dry MWCNT. Methods: C57BL/6J male mice were exposed to a daily average of ~60,000 particles/cm3 or breathing air for 4 hours/day, 5 days a week for 2 weeks. Mice were euthanized 3 and 14 days post-exposure for assessment of bronchoalveolar lavage (BAL) cellular profile, function and lung histology. Alveolar macrophages (CD11c+Autofluorescenthigh) were incubated with PE-labeled *E. coli* to assess phagocytic activity by flow cytometry. Aortic and mesenteric contractile and relaxation responses were assessed by wire myography. Results: Inhalation of MWCNT induced small increases in BAL total number of cells, mainly due to increases in macrophage numbers. Phagocytic activity was significantly reduced in alveolar macrophages recovered from MWCNT exposed mice compared to air controls at 3 days post-exposure. Lung histology revealed focal areas of subpleural thickening, increased cellularity and accumulation of MWCNT in alveolar macrophages throughout the lung. Vascular responses included modest reduction in acetylcholine mediated relaxation at 3 day post exposure which waned by 14 days post exposure. The maximal stress generated differences in vascular tissues were exaggerated by indomethacin. Conclusion: Inhalation of occupational levels of MWCNT leads to accumulation of MWCNT in alveolar macrophages and reduced phagocytic capacity, increased BAL cellularity, and pulmonary pathology. MWCNT inhalation produced modest changes in vascular tissue responses which was exaggerated with COX inhibition. Taken together these data allow us to suggest that exposure to occupational levels of MWCNT alters alveolar macrophage function and vascular reactivity that may contribute to heightened cardiopulmonary responses following subsequent challenge. Work supported by NIEHS U19ES019525, NIEHS R01-ES020897, NCBC grant 2013- BRG-1202 and ECU.

3508 Multi-Walled Carbon Nanotubes Down-Regulate Estrogen Receptor Alpha Gene Expression in Human Lung Epithelial Cells: A Potential Role for Transforming Growth Factor Beta1

L. C. Smith and T. Sabo-Attwood. *Physiological Sciences, University of Florida, Gainesville, FL.*

The role of sex hormones and their receptors in pulmonary fibrosis (PF) is unclear. PF is a fatal lung disease characterized by progressive and irreversible scar tissue formation leading to organ malfunction and respiratory failure. PF can have unknown etiology as in idiopathic pulmonary fibrosis (IPF) or be associated with exposure to environmental agents such as asbestos fibers and potentially multi-walled carbon nanotubes (MWNT) which have a superficial resemblance to asbestos. Sex-specific trends in the incidence and prevalence of IPF suggest that sex hormones and their receptors may be involved in PF. Crosstalk between estrogen receptor alpha (ESR1) and transforming growth factor beta1 (TGF-β1), a pro-fibrotic cytokine and major driver of idiopathic, asbestos-, and carbon nanotube-induced fibrotic signaling, occurs in breast cancer cells where TGF-β1 driven transcription factors down-regulate ESR1 gene expression. As such, the goals of this research were to investigate the potential for cross talk between ESR1 and TGF-β1 in response to exposure to MWNT in human lung cells. Using a human bronchial epithelial cell line (BEAS-2B), we have shown that ESR1 is the most abundantly expressed estrogen receptor isoform in our model and that exposure of BEAS-2B to 100 µg/ mL MWNT for 24 hours caused a 25 % decrease in ESR1 gene expression as determined by qRT-PCR. We further show that the decrease in ESR1 expression is concomitant to a 1.8 fold increase in TGF-β1 total protein levels as determined by ELISA. Additional experiments have shown that exposure of BEAS-2B to 1 ng/mL TGF-B1 is sufficient to down-regulate ESR1 gene expression by 45 % ($p < 0.05$). Collectively, these results suggest that MWNT down-regulate ESR1 expression through up-regulation of TGF-β1. Current studies are examining the downstream TGF-β1signaling pathways responsible for the observed reduction in ESR1 expression. This work is significant because it describes a previously unknown interaction between TGF-β1 and ESR1 in lung cells and suggests a protective role for ESR1 in fibrogenic signaling in response to MWNT and TGF-β1. Results from these studies will aid in refining diagnosis, assisting prognostic estimates, and identifying potential therapeutic targets of pulmonary fibrosis.

3509 Effects of Soap-Water Wash on Human Epidermal Penetration

H. Zhu, E.-C. Jung, C. Phuong, X. Hui and H. Maibach. *Dermatology, UCSF, San Francisco, CA.*

Skin decontamination is a primary intervention to decrease dermal absorption of hazardous contaminates, including chemical warfare agents, pesticides and industrial pollutants. Soap and water wash, the most common and readily available decontamination system, may enhance percutaneous absorption, namely "wash-in effect". To better understand the effect of soap-water wash on percutaneous penetration, and provide insights into improving skin decontamination, *in vitro* human epidermal penetration of four C14-labeled model chemicals—hydroquinone, clonidine, benzoic acid and paraoxon, was assayed using flow-through diffusion cells. Stratum corneum (SC) absorption rates of these chemicals at various hydration levels (0–295% of the dry SC weights) were determined and compared with the results of epidermal penetration to clarify the effect of SC hydration on skin permeability. Results showed accelerated penetration curves of benzoic acid and paraoxon after surface wash at 30 minutes post dosing. Thirty minutes after washing (60 minutes post dosing), penetration rates of hydroquinone and benzoic acid decreased due to reduced amounts of chemical on skin surface and in stratum corneum. At experiments end (90 minutes post dosing), soap-water wash resulted in lower hydroquinone penetration, greater paraoxon penetration, and similar levels of benzoic acid and clonidine penetration than non-wash groups. The observation of wash-in effect agrees with the enhancement effect of SC hydration on SC chemical absorption rate. These results suggest SC hydration derived from surface wash as one cause of wash-in effect; the occurrence of wash-in effect is chemical and delayed decontamination time dependent. By reducing the chemical residue on skin surface and in SC reservoir, soap-water wash may decrease chemical dermal absorption in the long terms; however the accelerated absorption of chemical toxins, especially chemical warfare agents, could be lethal.

3510 Investigation of Depth-Dependent Stratum Corneum Permeability in Human Skin *In Vitro*

H. Zhu[2], J. J. P. Cadavona[1], X. Hui[2], E.-C. Jung[2] and H. Maibach[2]. [1]*University of Nevada School of Medicine, Las Vegas, NV and* [2]*Dermatology, UCSF, San Francisco, CA.*

Stratum corneum (SC), a permeable membrane, limits the rate of percutaneous penetration. Since SC composition and structural properties responsible for skin barrier function appear depth-related, we conducted an *in vitro* dermatopharmacokinetic study on intact and tape stripped skin samples to clarify whether SC is a homogeneous barrier for chemical transport. SC concentration -thickness profiles were determined for four C-14 labeled model chemicals - panthenol, benzoic acid, paraoxon and butanafine, using tape stripping approach. Data analysis with the unsteady-state diffusion equation of Fick's second law permitted chemical diffusion coefficient in SC. To evaluate consistency of SC permeability from its surface to lower levels, skin samples were tape stripped 5 to 10 times to remove upper cell layers before chemical application, so that diffusion coefficients could be determined from three SC depth levels (0, 5 and 10 tape strips). Results suggested the depth-dependency of SC permeability to panthenol, benzoic acid and butenafine; diffusion coefficient of panthenol decreased significantly after the first 5 tape strips and remained consistent with further stripping. A progressive increase in diffusion coefficients of benzoic acid and butenafine was observed as tape stripping levels increased. Removal of superficial SC did not result in significant difference on paraoxon diffusion coefficient. For individual chemicals, variation of diffusion coefficient from SC surface to deeper layers agreed with the change of diffusion coefficient over time in intact skin. Characterization of SC properties contributing to the depth-dependent SC permeability will provide insight to skin penetration/decontamination.

3511 Modulation of Chemical Dermal Absorption by 14 Natural Products: A Quantitative Structure Permeation (QSPR) Analysis of Components Often Found in Topical Cosmeceuticals

F. Muhammad[1], M. Jaberi-Douraki[1], D. de Sousa[3] and J. E. Riviere[1]. *[1]Anatomy and Physiology, Kansas State University, Manhattan, KS; [2]Department of Mathematics, Kansas State University, Manhattan, KS and [3]Department of Pharmaceutical Sciences, Federal University of Paraiba, Paraiba, Brazil; [4]Institute of Computational Comparative Medicine, Kansas State University, Manhattan, KS.*

A large number of cosmetics and topical pharmaceuticals contain compounds of natural origin. There is a rising concern if these compounds can interact with the activity of other topically applied components in these formulations. The current study demonstrates modulation of dermal absorption of model components often found in cosmeceutical preparations (14C caffeine and 14C salicylic acid) by a set of 14 synthetic compounds of natural origin using a flow through *in vitro* porcine skin diffusion system. The parameters of flux and permeability were calculated and QSPR analysis conducted on different molecular descriptors of modulator compounds. Terpinyl acetate was the leading permeability/flux enhancer of caffeine followed by s-perilyl acetate and limonene 1, 2-epoxide. The permeability/flux of salicylic acid was highest with hydroxycitronellal followed by limonene 1, 2-epoxide and s-perilyl acetate. Step wise regression analysis for caffeine permeability/flux revealed polar surface area and log P while for salicylic acid, Henry's Law constant, number of freely rotatable bonds and water solubility as the optimum descriptors for prediction additive modulation. In parallel with the experimental techniques, a novel mathematical model was developed to estimate the permeability coefficients and improve the stepwise regression analysis for assessing modulator effects. R2 values increased from 0.70 for the QSPR model with three descriptors to 0.99 for the new model. Notably, limonene 1, 2-epoxide and s-perilyl acetate were excellent enhancers for both caffeine and salicylic acid. These results confirm that some natural products incorporated into topical formulations will enhance absorption of other components which could affect their safety and efficacy profiles. (Supported by Kansas Bioscience Authority)

3512 Partition and Diffusion Coefficient Determinations for Twelve Compounds in Isolated Human Skin Layers for *In Silico* Dermal Pen Modeling

J. D. Manwaring[5], C. Obringer[5], N. Hewitt[2], A. Schepky[1], D. Gerstel[1], M. Klaric[1], J. Eilstein[3], S. Gregoire[3], H. Duplan[4], C. Jacques-Jamin[4], R. Cubberley[7] and H. Rothe[6]. *[1]Beiersdorf AG, Hamburg, Germany; [2]Cosmetics Europe, Brussels, Belgium; [3]L'Oreal, Aulnay-Sous-Bois, France; [4]Pierre Fabre Dermo-Cosmetique, Toulouse, France; [5]Procter & Gamble, Mason, OH; [6]Procter & Gamble, Schwalbach, Germany and [7]Unilever, Sharnbrook, United Kingdom.* Sponsor: G. Daston.

The development of a precise *in silico* skin penetration model is needed to enable moving away from a 100% systemic bioavailability assumption to a more realistic one and to confidently assess the actual fraction of a compound systemically absorbed for finite dose scenarios. We are replacing empirical relationships (with limited applicability domains) with mechanistically based simulations to characterize trans-dermal transport. To this end, diffusion (D) and partition (K) coefficient determinations were conducted for several radiolabelled compounds in isolated human skin layers, which included for K determinations, measurements in isolated dermis, whole epidermis (stratum corneum (SC) plus viable epidermis), SC; and delipidized SC and SC lipids where appropriate. D determinations were made in dermis, whole epidermis and SC. As part of a Cosmetics Europe initiative, the first 12 compounds to be tested were benzoic acid, resorcinol, methyl paraben, dinitrochlorbenzene, acetophenone, 7-ethoxycoumarin, toluenediamine, vanillin, dimethyl phthalate, caffeine, diethyleneglycol mono butyl ether and thioglycolic acid. K determinations were made after overnight incubations of the tissue layer or lipid component in buffer solution spiked with the radiolabelled compound, and by subsequent direct measurements of the radioactivity in the tissue layer/lipid component vs. buffer sample. D determinations were made using a non-linear regression of the cumulative receptor fluid content of radiolabelled compound, fit to the solution of Fick's 2nd Law. For each of the tested compounds the K values for the dermis, whole epidermis, viable epidermis, fully hydrated SC and fully hydrated delipidized SC were all relatively similar, and were always about 3-4 fold lower than the values in partially hydrated SC, partially hydrated delipidized SC and in SC lipids. Conversely, the D values for each of the 12 tested compounds, for the dermis, were all much larger (about an order of magnitude or more) than the values for intact fully hydrated SC and whole epidermis.

3513 Characterization of Transdermal Pharmacokinetics of Ketoprofen in Sinclair and Göttingen Minipigs

A. Stricker-Krongrad[2], M. Zhong[2], H. Leigh[2], Y. Liu[1], Y.-H. A. Fu[1], J. Liu[2] and G. Bouchard[2]. *[1]KCAS, Shawnee, KS and [2]Sinclair Research Center, Auxvasse, MO.*

Our objective was to characterize the transdermal pharmacokinetics of a ketoprofen cream formulation in the Sinclair and Göttingen breed of minipigs. Minipigs have a fixed skin tightly attached to the subcutaneous tissues similar to that in humans, which makes them a preferable model for dermal studies. Three minipigs per gender per strain (9.1–9.9 months old) were used in the study. Four mL of 2.5% ketoprofen cream, (100 mg ketoprofen), were applied on an area on the back of each minipig that was approximately 3% of the total body surface area. Blood samples were collected at 4, 8, 12, 24, 28, 32 and 36 hours post-dose. The plasma samples were analyzed with API 4000 LC/MS/MS system with a stable d3-labeled ketoprofen used as the internal standard. The linear range of quantitation for ketoprofen was 50 to 25600 ng/mL. The PK parameters were calculated with PKSolver. Individual animal data were analyzed with the non-compartmental analysis. Transdermal pharmacokinetic parameters of ketoprofen were (N= 6; mean +/- SD; Sinclair versus Gottingen): t1/2 (h): 12.2 +/- 2.0 versus 11.7 +/- 3.9; Tmax (h): 8.0 +/- 2.5 versus 9.3 +/- 2.1 and Cmax (ng/m): 94.3 +/- 65.6 versus 173.1 +/- 71.8. Although ketoprofen was shown to be absorbed better in Göttingen than in Sinclair minipigs in the average plasma concentration time curves, no statistically significant difference was observed between all PK parameters. These data suggested that ketoprofen has a similar ADME process in both minipig lineages.

3514 New BPX-01 Formulations Improve *In Vitro* and *In Vivo* Delivery of Minocycline in Minipigs

D. Lac, M. Hermsmeier, D. Thomas, N. Yam, X. Chen, A. Yamamoto, S. Y. Huang, K. Chan and U. Nagavarapu. *Biopharmx, Menlo Park, CA.*

As many pubescent teenagers can attest, acne vulgaris is a chronic disease that can be very difficult to treat. The pathogenesis of this disease has been attributed to the presence of *Propionibacterium acnes* (*P. acnes*). A number of different treatments that exist can reduce the number of *P. acnes*, but such treatments often have been associated with the emergence of resistance in *P. acnes*. Currently, oral treatments are used, alone or in combination with topical products. Recently, a unique, stable, hydrophilic topical minocycline gel formulation BPX-01 was developed for the treatment of acne vulgaris. The absorption of such hydrophilic molecules into the skin typically occurs through pores or other openings in the skin (e.g. hair follicles). However, in order to achieve efficient delivery, appropriate solubilizing agents and solvent systems improve the efficiency of API delivery to target tissue/lesion. The purpose of this investigation was to determine if new BPX-01 formulations could improve *in vitro* skin penetration and *in vivo* delivery. The formulations were evaluated in minipigs to investigate the uptake of minocycline in skin. Our main aim was to observe drug related dermal toxicity after application of the formulations for over two weeks. Results show that the low dose formulation delivered a significant amount of active ingredient into the skin. This delivery was localized in the epidermis and pilosebaceous glands thereby indicating its potential to treat acnes vulgaris. In conclusion, minocycline was not detected in plasma, no irritation was observed, demonstrating its safety and effectiveness for the treatment of acne vulgaris.

3515 Human Skin Permeation *In Vitro* of Glycol Ether Mixtures and Ethanol Amines

N. B. Hopf[1], N. Charrière[1], C. Oltramare[1], P. Sping[2] and A. Berthet[1]. *[1]Institute for Work and Health, Epalinges, Switzerland and [2]Dermatology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.*

Skin is a major route of uptake of several substances used in cleaning products. Many are known to induce dermal irritation and sensitization. The culprit chemicals are often not known as cleaners are exposed to a multitude of substances simultaneously, making the exposure assessment complex. An *in vitro* flow-through diffusion cell system was used to study the potential effects of simultaneous exposures to glycol ethers (propylene glycol methyl ether (PGME) and propylene glycol n-butyl ether (PGBE)); and to known sensitizers such as ethanol amines (monoethanolamine (MEA)). All assays were performed using newly harvested human skin obtained from plastic surgery or previously frozen at -80°C. Different concentrations were applied to the dermatomed skin

mounted onto the system; PGME/PGBE mixture (50:50, 60:40, 75:25 % (v/v)), PGME and PGBE alone, and MEA (2% and 10% diluted in water. After 24 h exposure, skin was qualitatively assessed for irritation by histology. PGME permeated human skin greater (≈ 10 times) than PGBE both for fresh and frozen skin. The skin permeation coefficient (Kp) for PGME was similar for fresh and frozen skin (0.11 vs 0.37 cm/h), while greater for frozen compared to fresh skin for PGBE (0.004 vs 0.094 cm/h). The Kp for the mixture was greater (0.042) than for the two alone (0.11 cm/h for PGME and 0.022 to 0.17 cm/h for PGBE), suggesting an increase in PGBE dermal absorption but no change for PGME. Lag times were similar for PGME and PGBE (1.5 to 2.6h). MEA (aq) did not permeate skin below 5% dilution, and not before 6-7 h at higher concentrations (Kp = 0.26 to 1.31 cm/h). However, irritation was visible after only 15 min exposure to 1% MEA. For PGME and PGME, irritation damages were observed only for the 75:25% mixture. Cleaners are exposed to irritants and mixtures that can increase skin absorption compared to a substance alone, therefore future exposure assessments should incorporate this knowledge to avoid underestimating dermal exposures during cleaning tasks.

3516 A Novel Assay for Evaluating Wound Healing in a Full-Thickness *In Vitro* Human Skin Model

M. Bachelor, A. Armento, J. Oldach, G. Stolper, M. Li, M. Klausner and P. Hayden. *MatTek Corporation, Ashland, MA.*

Cutaneous wound healing involves interactions between dermal fibroblasts and epidermal keratinocytes as well as cell-extracellular matrix interactions. The current study describes wound healing experiments conducted in a full thickness *in vitro* human skin model (EpiDermFT). This model exhibits stratified epidermal components and a fully developed basement membrane and resembles *in vivo* skin in regard to both morphology and barrier function. Small epidermal only wounds were induced in the model using a 3mm punch biopsy and subsequently evaluated at various recovery time points by two methods. Historically, EpiDermFT has been used to evaluate re-epithelization of the wound by: a) manually bisecting the tissues through the center of the wound, b) staining with hematoxylin and eosin, and c) quantifying migration from the wound origin. Accurate bisection of the wound in difficult and often leads to variability in assay results. Here we describe a novel method of visualizing wound re-epithelization *in situ* simplifying analysis and reducing introduction of variables inherent in tissue processing that could potentially confound data. Following wounding, tissues were fixed and immunostained with markers of epidermal differentiation as well as a marker of fibroblasts allowing simultaneous visualization of migrating keratinocytes (keratin 14), differentiated suprabasal cells (involucrin), and dermal fibroblasts (vimentin) within the wound. Histological and immunohistochemical analysis showed keratinocyte migration at 2 days following wounding. In both methods, wounded tissues cultured without growth factors (2% human serum) had a reduced healing rate in which keratinocytes did not cover the entire wound within a 6 day timeframe. In contrast, wounded tissues cultured with growth factors demonstrated a dramatic increase in healing rate as keratinocyte migration completely covered the wounded area by day 6. In conclusion, this novel method of evaluating re-epithelization by utilizing immunohistochemical markers of differentiation is a quicker and more reproducible method of analyzing wound healing.

3517 Possible Substrate-Independent Role of N-Acetyltransferase 1 (NAT1) in Human Keratinocytes

J. Lichter and B. Blömeke. *Environmental Toxicology, University Trier, Trier, Germany.*

The human xenobiotic metabolizing enzyme N-acetyltransferase (NAT1) catalyzes the transfer of an acetyl group from acetyl co-enzyme A (AcCoA) to its substrates. Only one endogenous substrate is known and the physiological role of this enzyme is still elusive. NAT1 is expressed ubiquitously in all tissues analyzed so far including the skin. Although skin cells like keratinocytes, fibroblasts and dendritic cells are known to functionally express NAT1, it is not clear if this is also true for differentiated keratinocytes, which are the major part of the epidermis. In order to analyze NAT1 in differentiated keratinocytes, we used proliferating and calcium differentiated keratinocytes and found identical NAT1 mRNA levels and NAT1 enzyme activities in the analyzed states of differentiation. Since the process of differentiation is accompanied by a dramatic change in expression and cellular localization of proteins, we additionally stained NAT1 protein in those cells. In addition to the known expression of NAT1 in the cytoplasm, we found perinuclear staining in proliferating and similar staining intensities in nucleus as well as cytoplasm in calcium differentiated keratinocytes. Since the turnover of exogenous substrates by whole cells was identical in different states of differentiation, the role of NAT1 in the nucleus might be independent from substrate acetylation.

3518 Quantitative and Qualitative Assessment of Skin and Eye Phototoxicity in the Rat: Pigmented versus Non-Pigmented Strains

C. Gerbeix, S. Turnock, C. Thirion-Delalande and R. Forster. *CITOXLAB, Evreux, France.*

Pharmaceutical compounds that absorb light in the UV/Vis range and give a positive result in an *in vitro* assay such as the 3T3-NRU-PT assay must be further evaluated for phototoxicity using an *in vivo* assay, as specified in the newly published final version of the ICH S10 guidance. For compounds which reach the eye and absorb light above 400 nm, retinal phototoxicity must also be assessed. In addition ICH S10 guidance suggests the use of pigmented strains, based on the melanin-binding properties of the active principle to be tested. In the present study, pigmented Long-Evans (LE) and albino Sprague-Dawley (SD) rats were used to assess the phototoxic potential of sparfloxacin (SPX) and 8-methoxypsoralen (8-MOP), two known melanin-binding phototoxic compounds, using qualitative and quantitative endpoints. The study design was adapted from the UV-LLNA protocol which is in routine use in our facility. Groups of 5 rats received the test items by gavage for 6 consecutive days, accompanied by irradiation at 10J UVA/Vis on days 4 to 6. Local reactions and clinical signs were noted each day. On day 7, animals were sacrificed, ear biopsy and auricular lymph nodes were sampled and weighed, and eyes were also sampled for microscopic examination. The systemic phototoxicity of both chemicals was demonstrated in LE and SD rats, on the basis of lymph node weight, lymph node cell count and ear weight. Findings were generally more marked in albino SD rats. Histopathological findings of ocular phototoxicity were noted in the albino rats only, although ocular phototoxicity has not been reported in humans with these two chemicals. Our results with these two chemicals show that the UV-LLNA protocol in pigmented rats can provide a useful approach to determination of phototoxic potential and may provide a more relevant model for humans than albino models.

3519 Phototoxicity of Ethyl Vanillin: Comparison of Two *In Vitro* Methods

G. Ritacco and A. M. Api. *Research Institute for Fragrance Materials, Woodcliff Lake, NJ.*

Ethyl Vanillin (CAS # 121-32-4) is a widely used, aromatic aldehyde fragrance material which imparts an intense vanilla odor. It has a total systemic exposure of 0.083 mg/kg body weight (bw)/day. UV spectra for this material demonstrate absorption in the UVA/UVB region; with peaks at approximately 290 and 310 nm and absorbance returning to baseline at approximately 340 nm. The calculated molar extinction coefficient, for peak absorbance within the range 290-700 nm, is above the threshold of 1000 L • mol-1 • cm-1, indicating significant absorbance and the potential for photoactivation and possible phototoxic effects (Henry et al., 2009). Further testing was conducted to assess the phototoxic potential of the material. In a 3T3-Neutral Red Uptake phototoxicity assay (OECD 432) ethyl vanillin, with a mean phototoxic effect (MPE) ≥0.15, was predicted to be phototoxic. Follow up testing was performed. Ethyl vanillin was evaluated in human keratinocytes using the EpiDerm Skin Model (MatTek Corporation) in the presence or absence of UVA/visible light. The assay included a negative control, solvent control, and a positive control (chlorpromazine). Ethyl vanillin was tested at 3 concentrations - 1, 3 and 6% in polyethylene glycol (PEG). The tissues were subjected to UVA for 60 ± 2 minutes (6 J/cm2). For any dose, if the test material induces a 30% decrease in viability in the presence of UVA compared to viability in the absence of UVA, the material is considered phototoxic. Ethyl vanillin did not induce significantly reduced viabilities between UVA treated and untreated tissues, at any of the concentrations (1, 3, or 6%) and according to the guidelines of the assay, is not considered phototoxic. A human phototoxicity study demonstrates how the *in vitro* assay predictions compare with clinical data.

3520 A Complete Dataset Representing the Adverse Outcome Pathway (AOP) of Skin Sensitization for 20 Fragrance Allergens

R. Parakhia, D. O'Brien, J. Lalko and A. M. Api. *RIFM, Woodcliff Lake, NJ.*

The OECD developed an Adverse Outcome Pathway (AOP) for skin sensitization based on the mechanistic understanding of the biological key events leading to skin sensitization. This consists of four key events, the first three which are represented by *in vitro* assays. The OECD also approved the in chemico skin sensitization assay (DPRA) and ARE Nrf2 luciferase test method (KeratinoSens) for key events 1 and 2, respectively. Key event 3 is represented by the hCLAT and U-SENS assays; the former is currently under final stages of OECD approval. Key event 4 is evaluated by the *in vivo*, OECD approved, stand-alone Local Lymph Node Assay. An experimental dataset consisting of 20 fragrance allergens were tested using DPRA, KeratinoSens, hCLAT, and U-Sens, then compared with existing LLNA and HRIPT data. The data generated were analyzed for sensitivity of the individual assays and also in a 2 out of 3 approach proposed by others. The analysis shows that the sensitivity of an individual alternative method to predict sensitization varies greatly. Sensitizers were correctly predicted 45, 60, 80 and 50% of the time in DPRA, KeratinoSens, hCLAT and USens, respectively. However, when a 2 out of 3 approach is applied, 75% and 50% are predicted correctly with key event 3 represented by hCLAT or USens, respectively. We analyzed the OASIS TIMES software's ability to identify sensitizers among these fragrance materials. The highest sensitivity was observed with hCLAT followed by OASIS TIMES, KeratinoSens, U-Sens and DPRA respectively. In summary, the analysis supports the use of alternative methods in a WoE approach to identify skin sensitization potential of fragrance materials.

3521 Development of a New Local Lymph Node Assay That Evaluates the Elicitation Response

K. Yamashita[1], S. Shinoda[2], S. Hagiwara[2] and H. Itagaki[3]. *[1]Daicel Corporation, Himeji, Japan; [2]Drug Safety Testing Center Co., Ltd., Hiki-gun, Japan and [3]Faculty of Engineering, Yokohama National University, Yokohama, Japan.*

Introduction: We developed the LLNA modified by Daicel based on ATP content (LLNA-DA) which was adopted by the Organization for Economic Co-operation and Development (OECD) as test guideline (TG) number 442A (OECD, 2010). LLNA:DA evaluates skin sensitization potential of chemicals in induction phase like other LLNA methods. Thus, the two primary challenges regarding the use of the LLNA:DA remain. One is that some skin irritants could be classified as either positive or borderline positive. The other is that this assay could not evaluate the potential for cross-sensitization potential between chemicals, similar to the other LLNA methods. Aims: To develop a new local lymph node assay (LLNA) that evaluates the elicitation response to discriminate the true skin sensitizers from the other chemicals classified as borderline positive by LLNA:DA, .and to evaluate cross sensitization potential. Method: Using the new method, we tested 41 chemicals. Test group of mice were treated with the chemicals on the dorsum of their right ear on days 1, 2, and 3, and on the dorsum of both ears on day 10. Control group of mice were treated with the chemicals on the dorsum of the left ear on day 10. Lymph nodes were excised and weighed on day 12. If the difference in weights of the lymph nodes from the left ear between both groups was statistically significant, then we classified the tested chemical as a skin sensitizer. The results of 39 chemicals of 41 were consist with LLNA results. We tested 3 chemical pairs that were expected for cross-sensitivity such as p-benzoquinone and hydroquinone, and 2 pairs that were not expected for cross-sensitivity to evaluate the potential of this new method for cross sensitization testing. Results: Our results for all 39 chemicals, except nickel chloride and squalene, were consistent with those from LLNA. In addition, 3 of the 5 pairs showed clear cross sensitizing potential. Conclusion: The accuracy of this new method was 95% when compared to LLNA results. Furthermore, this new method showed the potential to evaluate cross-sensitization potential between chemicals. We conclude that this new assay, termed LLNA:DAE, has the potential for skin sensitization testing.

3522 Higher Sensitivity of the Abdominal Skin Than the Back Skin to Phototoxicity in Female Sprague-Dawley Rats

K. Kuga, H. Yasuno, Y. Harada, M. Hasegawa, Y. Miyamoto, M. Miyamoto and K. Sato. *Drug Safety Research Laboratories, Takeda Pharmaceutical Company Limited, Kanagawa, Japan.* Sponsor: M. Friedman.

The Sprague-Dawley (SD) rat is one of the most common strains used in wide variety of toxicity studies, while information about sensitivity to phototoxicity has been limited. The back skin has been commonly used for evaluation of phototoxicity in rats, but little is known about possible differences in sensitivity among various sites of skin. Therefore, we compared skin sensitivity between the abdomen and back to 4 phototoxic drugs (pirfenidone [PFD], 8-methoxypsoralen [8-MOP], doxycycline [DOX] and lomefloxacin [LMFX]), and 1 non-phototoxic drug (gatifloxacin [GFLX]) in combination with ultraviolet radiation (UVR, 18 J/cm2 as UVA and 0.3 J/cm2 as UVB, xenon lamp) in female SD rats. The vehicle was 0.5 w/v% methylcellulose solution. Macroscopic and microscopic examinations were performed from 0.5 to 72 hours after administration of UVR. As a result, the abdominal skin showed clear phototoxic reactions after PFD administration followed by UVR, but the back skin did not elicit any findings associated with phototoxicity of PFD. In addition, severer and/or more frequent phototoxic reactions were demonstrated in the abdominal skin compared to the back skin after administration of 8-MOP, DOX and LMFX with UVR. Concentrations of PFD, DOX and LMFX in the abdominal skin were equivalent to those in the back skin. The vehicle control and GFLX did not induce any phototoxic reactions in either of the skins. Based on these results, we conclude that the abdominal skin of female SD rats is more sensitive to phototoxicity than the back skin. This model is useful for an *in vivo* phototoxicity study.

3523 Derivation of a Dermal Sensitization Threshold for Hexavalent Chromium in Textiles

B. Wang and M. H. Whittaker. *ToxServices LLC, Washington, DC.*

Hexavalent chromium (Cr (VI)) is a potent dermal sensitizer found in a variety of materials and products such as cement, leather, electronics, cosmetics and household cleaning agents. The prevalence of Cr allergy is about 3–4% in North America and Europe, and more than 16% in Asia in clinical studies. The purpose of this project is to perform a literature review and to derive a dermal sensitization threshold for Cr (VI) in textile materials. Thresholds for the induction of skin sensitization in naïve individuals derived from mouse local lymph node assays are 1–10 µg/cm2 skin, while thresholds for the elicitation of reactions in sensitized human subjects are much lower. The minimum elicitation thresholds in 10% of individuals allergic to Cr (MET10%) vary from 0.02 to 1.04 µg/cm2/2 days. Various regulatory bodies around the world adopted different threshold levels for products containing residual Cr (VI): Korea FDA and National Institute of Toxicological Research used an induction threshold of 1 µg/cm2 derived from an LLNA as the limit for Cr (VI) residuals in cosmetics; the U.S EPA used an MET10% of 0.092 µg/cm2 derived from a repeated open application test (ROAT) as the critical study for surface-treated wood; the EU used the lowest (hence most conservative) reported MET10% of 0.02 µg Cr/cm2 as the derived minimum effect level (DMEL) for leather materials that come into contact with skin; the Danish EPA considered an MET10% of 0.09 µg/cm2 the critical value for their risk assessment of Cr in leather shoes because this study has the highest number of test subjects. As the limit of detection of Cr in leather of 0.45 µg/cm2 (3 mg/kg) is much higher than the DMEL, the EU proposed that Cr (VI) be restricted to below limit of detection. While the MET10% values of 0.02 and 0.09 µg/cm2 can be used in risk assessments for Cr(VI)-containing textiles with and without prolonged skin contact, they may fall below the limits of detection (LODs) in practicality. In such situations, LODs should be used as thresholds for dermal sensitization risk assessment, which appear to be protective of induction, but not elicitation, of Cr(VI) sensitivity.

3524 Histological Differences in Skin of Minipigs Related to Age, Sex and Sample Site

J. Løgsted, G. Jeppesen, N. Grand and A. Makin. *CiToxLAB Denmark, Lille Skensved, Denmark.*

Minipigs are routinely used in testing of drugs intended for dermal application. The skin of the minipig is widely regarded as being the closest in structure to that of humans. In the design of studies it is important to ensure that the site used for dosing is as uniform as possible. If the site chosen is of variable thickness, or with a variable density of hair follicles, this potentially can affect penetration and therefore systemic exposure of substances. Typically in studies, minipigs are dosed on 10%

of their surface area, the site chosen being the sides and back of the animal where the skin is fairly uniform. We are from time to time challenged to use other areas or larger areas, therefore we have studied the differences in the skin between various sites on minipigs of different ages. The animals used were between 1 week and 9 months of age and were of both sexes. The areas chosen for evaluation were back, abdomen, groin/inguinal area and neck immediately behind the ears. The samples were evaluated histologically using the histopathological standard terminology to describe any changes in the slides. Furthermore, we recorded the number of hair follicles, the epidermal thickness (measured as average number of cells from three representative epidermal locations/slide) and the degree of keratinization (stated as low, medium or high). These measures were used to compare the different locations within the same pig as well as between the pigs. The results show that there are differences between the various sites and that in toxicology studies a dosing area of 10% of the body surface on the sides and back is suitable and uniform.

3525 The Cyanide Blues: Laser Irradiation of the Tattoo Pigment Phthalocyanine Blue Can Cause Formation of Highly Toxic Hydrogen Cyanide in Skin

I. Schreiver[1], C. Hutzler[1], N. Röder[1], P. Laux[1], H.-P. Berlien[2] and A. Luch[1]. [1]*Department of Chemical & Product Safety, Federal Institute for Risk Assessment, Berlin, Germany and* [2]*Department of Laser Medicine, Evangelical Elisabeth Hospital, Berlin, Germany.* Sponsor: A. Haase.

Toxicological properties of tattoo inks and permanent make-up have been barely investigated despite an apparently unstoppable popularity of this life-long body modification. During the last decades, colored inorganic pigments were mostly replaced by organic pigments with higher color brilliances. Hence, they are put at risk for being metabolized and photo-chemically cleaved by sunlight or laser irradiation when removed from the skin at the end of their dermal life. We here investigated the photochemical cleavage of the widely used organic pigment copper phthalocyanine blue upon Q-switched laser irradiation in aqueous suspensions and pig skin. Irradiated specimen were extracted with ethyl acetate and analyzed with gas chromatography coupled to mass spectrometric detection using liquid injection and head-space sampling techniques. 1,2-Benzenedicarbonitrile is formed as the main decomposition product in all experimental setups. Additionally, the highly toxic gas hydrogen cyanide and the human carcinogen benzene were formed in dependence of the laser wavelengths used and the initial pigment concentration. Cyanide levels of ≥1 mM evolving upon ruby laser irradiation of >1.5 mg/ml aqueous suspensions were proven to significantly reduce cell viability in human skin cells *in vitro*1. Comparing this outcome with recently published pyrolysis data on copper phthalocyanine, high temperatures exceeding 600°C inside the pigment particles can be expected. Hence, all organic pigments bear the risk of releasing toxins and carcinogens upon laser induced combustion and removal and by that may harm the skin locally and even systemically due to full bioavailability of the compounds. Reference: 1Schreiver, I., Hutzler, C., Laux, P., Berlien, H. P. & Luch, A. Formation of highly toxic hydrogen cyanide upon ruby laser irradiation of the tattoo pigment phthalocyanine blue. Sci Rep 5, 12915 (2015).

3526 Inhibition of CRD-BP to Overcome Resistance to BRAF-Inhibitors in BRAF-Mutant Melanoma

T. Kim, Y. G. Xu, V. Selaturi and V. S. Spiegelman. *Dermatology, University of Wisconsin-Madison, Madison, WI.* Sponsor: C. Bradfield.

Melanoma is the sixth most common cancer in the United States and malignant stages of melanoma result in low survival rate. BRAF is the most frequent mutation in human melanoma with the majority being V600E (80%). Vemurafenib (PLX4032) is one of the FDA approved drug targeting V600E-mutant melanoma by inhibiting enzymatic activity of BRAF. Decreased phosphorylation on BRAF results in suppressed signaling to the downstream of MAPK pathways. Treatment with vemurafenib extended progression-free survival by 6 months after initial treatment; however the majority of the patients relapses and succumbs to melanoma that acquired resistance to vemuraefnib. Although combination therapies have been studied, secondary resistance may occur because multiple mechanisms can be involved in resistance to BRAF inhibitor. Here, we propose an approach, independent on the mechanisms of acquired resistance, by targeting a molecule critical for regulation of multiple pro-survival pathways in melanoma - Coding Region Determinant-Binding Protein, CRD-BP. CRD-BP is a messenger RNA binding protein that binds to various oncogenic targets such as βTrCP1, c-myc, Gli1, MDR1 and MITF. Binding of CRD-BP affects mRNA translation, localization and stability leading to increased survival, inhibition of apoptosis and development of drug resistance as well as induction of tumorigenic properties. We hypothesized that inhibition of CRD-BP can potentiate the action of BRAF inhibitors and aids in overcoming resistance to BRAF inhibitors. Our study shows that combination of BRAF inhibition and CRD-BP knockdown inhibits tumorigenic properties in metastatic melanoma such as reduced cell growth and cell proliferation as well as induction of G1 cell cycle arrest and apoptosis. In addition, inhibition of CRD-BP on vemurafenib-resistant cell line effectively attenuates the growth with treatment of vemurafenib. These findings indicate that CRD-BP may work as a novel target for BRAF-mutant melanoma.

3527 Assessing Physiological Response to Toxic Industrial Chemical Exposure in Megacities

D. L. Ippolito[2] and B. C. McDyre[1]. [1]*ORISE, Ft. Detrick, MD and* [2]*US Army Center for Environmental Health Research, Ft. Detrick, MD.* Sponsor: D. A. Jackson.

As military operations shift toward megacities, the risk of exposure to toxic industrial chemicals increases for deployed personnel. In this study, we identify organ injury biomarkers using a systematic review of the literature and bioinformatics data mining. An environmental scan and systematic literature review identified publications on environmental threats in a megacity combat environment using Web-based literature databases. We identified the top 30 megacity chemical threats by a probability assessment of toxicity and geographic distribution, then identified health effects and biomarker candidates by systematic literature review. Plasma microRNA expression was determined by qPCR in rodents orally exposed to allyl alcohol or carbon tetrachloride for 5, 14, or 28 days. Literature scan results indicated that most megacity threats related to air quality, physical injuries, chemical/radiation exposure, water quality, and infectious diseases (134 total papers). Lab-on-a-chip was the most common device for biomarker detection (34 publications). Lung injury was the most significant threat, followed by heart and liver damage. Plasma biomarkers of prognostic value include intercellular adhesion molecule-1, interleukins 6 and 8, plasminogen activator inhibitor-1, protein C, receptor for advanced glycation endproducts, surfactant protein D, von Willebrand Factor, and miR-125a-5p and 122. Differential serum miR-122 expression correlated with hepatotoxicity in rodents orally exposed to hepatotoxicants (5-18-fold; $p<0.05$). Early predictive biomarkers can be adapted to ruggedized, fieldable detection systems for aiding clinical decision-making in theater. Disclaimer: This research was supported in part by an appointment to the Postgraduate Research Participation Program at the USACEHR administered by the ORISE through an interagency agreement between the US DOE and USAMRMC. Research was conducted in compliance with the Animal Welfare Act and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the U.S. Army.

3528 Abstract Withdrawn by Author.

3529 Development and Validation of Five GyroMark HT Rat Kidney Toxicity Assays

B. Gilliam, C. Fry, S. Suchyta, J. Mistry and Q. Xiao. *EMD Millipore, Saint Charles, MO.* Sponsor: R. Wiese.

Organ toxicity is the leading cause of drug failure. Because traditional tests may lack specificity in identifying where damage is occurring and often cannot detect it until it is irreversible, more sensitive, rapid methods are needed to identify site-specific damage as early as possible. To identify kidney injury caused by various compounds, it might be necessary to screen nephrotoxicity biomarkers, often necessitating some level of automation and/or high throughput. The Gyrolab xP and xPlore workstations provide automated compact disc-based nanotechnology using microfluidics for sandwich ELISA immunoassay analysis, requiring a biotinylated capture antibody and a fluorescence labeled detection antibody. Gyros technology features small sample volume requirements (200–1000 nL), high sensitivity, broad dynamic range, and short assay time (1 hour per assay). EMD Millipore's GyroMark HT Rat Kidney Toxicity assays can be used for quantification of the following analytes in rat urine samples: β 2-Microglobulin, Clusterin, Cystatin C, Kidney Injury Molecule-1 (KIM-1), and Lipocalin-2/Neutrophil Gelatinase-Associated Lipocalin (NGAL). Assay sensitivities are 0.47 ng/mL, 0.34 ng/mL, 0.012 ng/mL, 0.01 ng/mL, and 0.021 ng/mL, respectively. The assay recoveries range from 93% to 106%, dilution linearity between 103% and 108%, intra-assay variation <10% and inter-assay variation <15%. Bead-based Milliplex assays and commercial ELISAs correlate well (R=0.94-0.99)

when evaluated against the GyroMark HT assays. Elevated levels of all analytes were detected in the urine of vancomycin- and gentamicin-treated rats compared to urine samples from water-treated rats. Our stringently validated rat kidney toxicity assays are highly robust and reproducible, making them useful tools for drug developers and basic researchers interested in detecting kidney injury in rat urine samples.

3530 Optimization of Intrathecal Administration in the Rat

G. Iacono, J. Douville, C. Foucault, R. St-Jacques, F. Emond and C. Copeman. *Charles River, Montreal, QC, Canada.* Sponsor: M. Vezina.

Over the past few years, there has been increased interest in using the intrathecal route of administration for targeted delivery of molecules to the central nervous system (CNS). This approach is more challenging in rodents than in large animals due to their small size and associated dose volume limitations. Refinement of the surgical techniques, including improving the rate of adequate catheter placement and reduction in the stress/pain related to the puncture of the access port is an imperative. Our laboratory has modified the surgical procedure by introducing the use of Instech PinPorts for delivery of agents in Sprague Dawley rats. PinPorts are designed to allow the exteriorization of the catheter while maintaining a closed system, therefore avoiding puncturing through the skin for each test material administration and reducing the pain and stress caused to the animal during manipulations. In addition, these access ports allow reduction of the overall volume to be injected due to their minimal dead volume. After surgical placement of the catheter and port, the animals were kept for an observation period of up to 14 weeks. Clinical signs, body weights and clinical pathology parameters were monitored and patency of the catheter system was also confirmed by injecting 0.02 mL of 0.9% Sodium Chloride for Injection, USP at least once weekly. The animals were then euthanized and the placement of the catheter was confirmed. The rate of success for the placement within the intrathecal space was 88% and patency was maintained for all animals throughout the observation period. The bodyweight of the animals was unaffected over the observation period and there were no adverse clinical signs observed. The clinical pathology evaluated was also within historical data at our laboratory. Overall, this demonstrates that the use of Pinports allows successful intrathecal administration in rats.

3531 Pilot Evaluation of a Novel Fully-Implantable Digital Telemetry Manometer: Acute *In Vivo* Responsiveness and Characteristics

C. L. del Rio[2], S. Roof[2], E. Ferris[2], A. Keel[2], B. Youngblood[2], J. Burton[1], H. Lan[1], J.-G. Napoléoni[1] and R. L. Hamlin[2]. [1]*EMKA Technologies, Paris, France and* [2]*QTest Labs, Columbus, OH.* Sponsor: R. Hamlin.

Introduction: Despite their invaluable role in toxicological and/or safety pharmacology studies, the availability of implantable radio-telemetry devices allowing the assessment of cardiovascular end-point, such as systemic/ventricular pressure and/or bio-potentials, remains limited. EMKA Technologies has developed a new fully-implantable device/system (easyTEL-D) that seeks to address these needs. This pilot study tested/verified the acute functionality and responsiveness of the sensing units embedded in such novel radio telemetry unit (easyTEL-D) both *ex vivo* and *in vivo*. Methods: In short, terminally anesthetized (isoflurane) New Zealand-White rabbits were used to evaluate the acute functional responses of the easyTEL unit to pharmacologically-induced chances in arterial pressures, chronotropy, and ECG morphology. In addition, the signal characteristics and responsiveness of the easyTEL unit were compared against those obtained from a previously implanted well-established telemetry unit/system (TL11M2-D70-PCT, DSI). Frequency-responses and signal characteristics were also evaluated *ex vivo*. Results: The easyTEL pressure transducer was sensitive to acute pharmacologically-induced vasopression (phenylephrine) and vasorelaxation (sodium nitroprusside) *in vivo*, presenting comparable responses to those recorded from an established implantable device. The easyTEL-D system was easily deployed, presenting negligible environmental interference and extended the dropout-free transmission distance by ~10 fold (I.e., 11.4 ± 1.9m vs. 1.1 ± 0.1m for the TL11M2-D70-PCT). Conclusion: Taken together, these data suggest that EMKA Technologies's new fully-implantable telemetry device/system (easyTEL-D) holds promise as a potential tool for the assessment of cardiovascular end-points in toxicology/pharmacology studies; however, both chronic and in-life performance remain to be evaluated.

3532 Toxicokinetic Analysis of Acetaminophen following Liquid Microsampling–Evaluations of Rat and Mouse Plasma and Serum and Impact of Collection Site

S. Ploch, L. Geiger, P. Mielke, D. Bilgo, C. Scheuerell and A. Ledvina. *Covance Laboratories, Inc., Madison, WI.*

Interest in bioanalysis of dried blood spots has diminished somewhat due to substantial regulatory and technical challenges (e.g. effects of hematocrit on bioanalysis); therefore the use of small liquid samples (liquid microsampling) has been gaining popularity for nonclinical toxicokinetic (TK) analysis. The benefits of small sample volume collections are particularly striking in mouse studies including transgenic strains where reductions in the numbers of satellite TK animals may provide a substantial ethical and cost benefit. Collection of microsamples for processing to serum (as compared to plasma) has also been a challenge when using glass capillary-based techniques. In this study, we evaluated a novel collection tube (Minivette® POCT, Sarstedt) with internal piston for dispensing for the collection and processing of 50 µL whole blood to either plasma or serum for analysis of acetaminophen toxicokinetic profiles in female rats [Crl:CD(SD)] and mice [two strains were used, Crl:CD1(ICR) and Ras H2 (Hemizygous)]. Rats and mice were administered a single gavage dose (150 mg/kg for rats and 100 mg/kg for mice) of acetaminophen. Rats were bled at approximately 0.5, 2, 4, 8, and 24 hrs postdose, from a tail vein for the microsample (50 µL) and a jugular vein for standard sample volume collections (0.5 mL). Mice were serially bled approximately 0.5, 4, and 24 hrs postdose from a saphenous vein (50 µL). Samples were processed to serum or plasma and evaluated for acetaminophen using LC-MS/MS. Exposure to acetaminophen in female rats was similar between serum and plasma matrices and between jugular vein and tail vein collection sites. Exposure to acetaminophen in female mice was similar between serum and plasma matrices and between Crl:CD1(ICR) and Ras H2 (Hemizygous) strains. These results indicate that this microsample blood collection method using Minivettes allows for evaluation of toxicokinetic exposure in both plasma and serum and is not impacted by the site of collection in the rat.

3533 Automation of Cytokinesis-Block Micronucleus Assay using ImageStreamX® Imaging Flow Cytometry

C. E. Probst[1], M. A. Rodrigues[1], L. A. Beaton-Green[2], B. C. Kutzner[2] and R. C. Wilkins[2]. [1]*Amnis Biology R&D, EMD Millipore, Seattle, WA and* [2]*Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, ON, Canada.* Sponsor: S. Casinghino.

Background: The cytokinesis-block micronucleus (CBMN) assay is used to measure chromosome damage by enumerating the number of micronuclei in binucleated cells, and has been successfully applied for *in vitro* studies of molecular toxicity as well as biodosimetry of individuals exposed to radiation. A major bottleneck for the conventional CBMN assay is that slides must be manually scored under a microscope by trained personnel, limiting its applicability for large-scale pharmaceutical screens or radiation incidents. While automated and semi-automated microscopy has been successfully used for the CBMN assay, slide preparation and scanning steps remain time consuming. Here we describe the development of a high-throughput, automated CBMN assay using the ImageStreamX imaging flow cytometer (CBMN-ISX). The ImageStreamX is a high-speed microscope that collects brightfield, side-scatter, and fluorescent imagery for objects in suspension at rates up to 1,000 objects per second. Methods: Using a suspension-based CBMN protocol, human blood was irradiated using a cabinet X-Ray machine with known doses from 0-4 Gy to generate a dose-response curve, which was then used to predict dose in 9 blinded samples. Brightfield and Draq5-labelled DNA imagery were collected for a minimum of 50,000 events for each sample using ImageStreamX, and an analysis template was developed in IDEAS image analysis software to quantify the number of micronuclei per binucleated lymphocyte. Results: The results demonstrate the CBMN-ISX assay generates dose-response curves of similar magnitude and shape to those previously reported for automated and semi-automated methods, and accurately predicted dose in all blinded samples within 0.5 Gy. Conclusions: The novel CBMN-ISX addresses throughput limitations of the CBMN assay while retaining high performance, and may thereby enable large-scale studies of cell cytotoxicity and patient biodosimetry.

3534 Utilizing High Throughput Flow to Screen for Compound Immunotoxicity in Peripheral Blood Mononuclear Cells

S. Perschke[2], Z. Liu[2] and K. Luu[2]. [1]*IntelliCyt Corporation, Albuquerque, NM and* [2]*Assay Development, IntelliCyt Corporation, Albuquerque, NM.* Sponsor: M. Misra.

The search for more predictive models of compound immunotoxicity has driven interest in the screening of primary cell models. While it is well appreciated that screening data from primary cells such as peripheral blood mononuclear cells (PBMCs) are more indicative of true compound or drug toxicity, the use of PBMCs in a screening assay context presents a unique set of challenges. Primarily, the limited availability and high costs of cells can be prohibitive to their usage in screening campaigns. Further, PBMCs are comprised of a mixture of immune cells. While this cell model is able to recapitulate many of the complex responses of the immune system to compound treatment, the model does also require a mulitparameter approach to be able to simultaneously detect cell phenotypes and functional readouts. Here we detail the usage of the iQue Screener (IntelliCyt) to detect differential sensitivity and toxicity of PBMC subsets to a small set of immunosuppressive compounds, and to generate multiplexed compound profiles for each compound. To characterize the performance of the multiplex high throughput assay in immune response profiling, a small set of immunomodulatory compounds were tested. These compounds include resveratrol, mitomycin C, imiquimod, methotrexate, myrocin, dexamethasone, thalidomide, and cyclosporine. Mannitol was used as a negative control for immune suppression as it is a common carrier in many drug formulations. PBMCs were stimulated to proliferate by the use of phytohemagglutinin (PHA), and the ability of each compound to suppress the proliferation of the various cell types in the mixed PBMC population was measured in 12-point dose response. The proliferation profile in the total cell population, and as well as T-cell subopulations were compared. From a single sample, we were able to immunophenotype by the use of α-CD3 and α-CD8 antibodies which enabled the identification of T-cells and cytotoxic T-cells from the mixed population. We were also able to simultaneously evaluate compound immunotoxicity and compound immunosuppression for each cell type. This data demonstrates the application of the iQue Screener platform to multiplex complex biology with primary cells in a suspension screening format, in a streamlined no-wash assay workflow.

3535 Refinement of a Commercially Available Multiplex Cytokine Kit to Enable Performance for Measurement of Göttingen and Sinclair Minipig Plasma

M. Doyle-Eisele, C. Tudan, L. Herrera, G. Degido, E. Maloy and J. Wilder. *LRRI, Albuquerque, NM.*

Cytokines act as early surrogate biomarkers for tissue damage following various challenges and have been shown to correlate with the risk of clinical toxicity, and persistent elevation has proven to mediate the cellular response to ionizing radiation (for example), making them important early surrogate biomarkers. One of the challenges in the use of cytokines as biomarkers is the homology in response among species and the ability of commercially available assays to be used for their measurement. Multiplex immunoassays allow the simultaneous analysis of multiple secreted cytokines, also making this technology very useful in pre-clinical studies, especially when volumes are limited. The goal of the research described here was to characterize the commercially available kit, and later to optimize it to meet acceptable performance standards. First, our studies corroborated the work reported by others that the commercially available porcine kits were not suitable for measurement in MP using the kit instructions due to failures in assay performance as determined by parallelism and dynamic range when comparing the MP plasma-associated curves against the kit-based porcine serum curve. We therefore sought to optimize the assay for each MP species by assessing the effects of diluting the samples with assay buffer, varying the amount of bead-immobilized antibody, and quantitating extended calibration curves against curves prepared under the multiple conditions investigated so as to provide an array of conditions that can be processed. Our modifications enabled us to extend the calibration curve ranges for all cytokines in both Gottingen and Sinclair MP plasma and generate analytical data meeting the acceptance criteria necessary for large molecule bioanalysis based on the 2012 EMA Guidance, and the draft 2013 FDA guidance on Bioanalytical Method Validation. (Work was supported under contract with the Biomedical Advanced Research and Development Authority (BARDA) Contract No. HHSO100201100004I, Task Order Number HHSO10033001T).

3536 Predictivity of *In Vivo* Metabolomics in Rats as Compared to Classical Toxicological Assessments

H. G. Kamp[4], O. Duerr[4], E. Fabian[4], F. Faulhammer[4], M. Freriks[4], M. Herold[3], G. Krennrich[4], G. Montoya[4], R. Looser[3], W. Mellert[4], E. Peters[3], V. Strauss[4], T. Walk[3] and B. van Ravenzwaay[4]. [1]*Metanomics GmbH, Berlin, Germany;* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany;* [3]*Experimental Toxicology and Ecology, Metanomics GmbH, Berlin, Germany and* [4]*Product Safety, BASF SE, Ludwigshafen am Rhein, Germany;* [5]*Quantum Chemistry, BASF SE, Ludwigshafen am Rhein, Germany.*

BASF SE and metanomics GmbH developed the *in vivo* metabolome data base MetaMap®Tox containing metabolome data for more than 550 reference compounds. Over the last several years, within the context of regulatory systemic toxicity testing (OECD 407, 408 and 422), an increasing number of metabolome analysis was performed on compounds with unknown toxicity. We present the evaluation of rat plasma metabolome analysis of 82 compounds, including 64 new substances and 18 pharmaceuticals (which were tested for the development of the data base). The results of the evaluation of the metabolome analysis (toxicity prediction) were compared against the outcome of classical parameters. This comparison was done for each of the compounds, separated by sex. Overall, 83% of the effects observed with classical parameters were correctly predicted by the evaluation of the metabolome data. In male animals 36 out of 79 compounds showed effects in classical parameters. Out of these, for 31 compounds (86%) the metabolome analyses correctly predicted such effects. Five out of these 38 compounds (14%) were found to be false negative. 43 out of 79 compounds did not show any effect in the classical parameters. Out of these, 37 substances (86%) also did not show an effect on the metabolome (correctly negatively predicted), whereas 6 compounds (14%) indicated effects based on the metabolome analysis.(false positive predictions). In females, 82 compounds were evaluated. Based on the metabolome evaluation, the toxicity of 29 out of 38 compounds was correctly predicted (76%); 9 out of 38 were false negative predicted (24%) For compounds without toxicity, 37 out of 44 were correctly predicted (84%), and 7 out of 44 were false positive predictions (16%). These results confirm that a prediction of rat toxicity using metabolome data can be used to assess compounds at an early stage of development (with few toxicological data).

3537 High-Throughput Biomonitoring for Exposome Research

D. P. Jones[1], Y.-M. Go[1], D. I. Walker[1], K. Uppal[1], K. Pennell[2] and G. W. Miller[1]. [1]*Emory University, Atlanta, GA and* [2]*Tuffs University, Medford, MA.*

The exposome is the cumulative measure of environmental influences and associated biological responses throughout the lifespan, including environmental exposures, diet, behavior, and endogenous processes. A major challenge for exposome research lies in the development of robust and affordable analytic procedures to measure exposures and associated biologic response occurring over a lifetime. The present research examined quantification strategies to enhance the usefulness of metabolomics profiling using liquid chromatography coupled to ultra-high resolution mass spectrometry. The results of metabolomics analyses on 157 human plasma samples show that biological effects can be linked to environmental exposures through metabolome-wide association studies (MWAS).The results also establish the feasibility of a reference standardization protocol in which unknown, individual chemical concentrations in human plasma samples are determined by comparisons to a concurrently analyzed, well-characterized pooled reference sample. Of those environmental chemicals quantified, octylphenol (57 ± 61 nM) and dipropylphthalate (60 ± 24 nM) were present at highest concentrations. Dibutylphalate was present in all samples, but calibration of standards was inconsistent. Styrene, flame retardants (triethylphosphate, triphenylphosphate), and some insecticides and related metabolites were also detected in the nanomolar range. Pirimicarb was present in most samples at sub-nanomolar concentration. The subjects studied were all non-smokers and measured values of cotinine (4.6 ± 4.2 nM; median 4.0 nM) were similar to human metabolome database (HMDB) values for non-smokers. Caffeine, which is directly derived from diet, and hippurate, which is formed by glycine conjugation of dietary benzoic acid, were present with micromolar concentrations. The results establish a simple protocol for quantitative biomonitoring that will enhance data collection and population screening in exposome research.

3538 Computational Models of the Aryl Hydrocarbon Receptor PAS-B Domain: Successful Applications in Cancer Prevention, Treatment and Predictive Toxicology

W. H. Bisson[1], G. Perdew[2] and R. L. Tanguay[1]. [1]*Oregon State University, Corvallis, OR and* [2]*Pennsylvania State University, University Park, State College, PA.* Sponsor: R. Tanguay.

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that regulates the expression of a diverse group of genes. Exogenous AHR ligands include the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is a potent agonist, and the synthetic AHR antagonist N-2-(1H-indol-3yl)ethyl)-9-isopropyl-2- (5-methylpyridin-3-yl)-9H-purin-6-amine (GNF351). As no experimentally determined structure of the ligand-binding (PAS-B) domain exists, homology models have been utilized for virtual ligand screening (VLS) and molecular simulations. We recently developed an "agonist-optimized" homology model of the human, mouse and zebrafish AHR PAS-B domain. The models were successfully applied for the characterization of AHR ligands, to gain insight on the evolutionary variation in the mechanics of the AHR PAS-B domain and to predict the toxicity of ubiquitous environmental chemicals. The comparison of the data generated from the different species of the AHR is useful for understanding the evolutionary role of polarity, shape, 3D-location of specific residues in the PAS-B domain for biological activity. The results obtained will help future design and development of novel AHR ligands for cancer prevention and treatment. Metabolism of PAHs present in the environment, poses an unknown risk to human health. Combining 22 different developmental endpoints in the zebrafish model and docking scores from human and zebrafish *in silico* AHR models, we predicted and correlated *in vivo* AHR-induced toxic effects for most of the synthesized and commercially available nitro-(NPAHs) and oxo-metabolites (OPAHs) included in the study. The AHR models were also recently included in the TOXCAST program for the *in silico* screening of a large database of chemicals and marketed drugs to predict AHR activity. The National Research Council Report Toxicity Testing in the 21st century (TT21c) called for embracing new technologies and basing assessments on toxicology mechanisms related to human biology and exposure. Computational models of the AHR PAS-B domain, in combination with multiple *in vitro* and *in vivo* zebrafish assays, represent a useful tool for cancer prevention, treatment and predictive toxicology.

3539 Linking High Resolution Mass Spectrometry Data with Exposure and Toxicity Forecasts to Advance High-Throughput Environmental Monitoring

J. E. Rager[4], M. J. Strynar[3], S. Liang[4], R. McMahen[4], A. M. Richard[3], C. M. Grulke[1], J. F. Wambaugh[3], K. K. Isaacs[3], R. Judson[3], A. J. Williams[3] and J. R. Sobus[3]. [1]*Lockheed Martin, Research Triangle Park, NC;* [2]*NCCT, US EPA, Research Triangle Park, NC;* [3]*NERL, US EPA, Research Triangle Park, NC and* [4]*US EPA, ORISE, Research Triangle Park, NC.*

There is a growing need for rapid chemical screening and prioritization for regulatory decision-making. Here we describe a study that links high resolution mass spectrometry (HRMS) data with exposure predictions from the US EPA's ExpoCast program and *in vitro* bioassay data from the Tox21 consortium. Vacuum dust samples were collected from 56 households across the US. Sample extracts were analyzed using liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS) with electrospray ionization. On average, ~2000 molecular features were identified per sample in negative ion mode, and 3000 in positive ion mode. Exact mass, isotope distribution, and isotope spacing were used to match molecular features with a unique listing of chemical formulas extracted from EPA's Distributed Structure-Searchable Toxicity (DSSTox) database. A total of 978 formulas were consistent with the dust molecular feature data; these formulas mapped to 3228 possible chemicals in the database. Correct assignment of a unique chemical to a given formula required additional validation steps. Each suspect chemical was prioritized for follow-up confirmation using abundance and detection frequency results, along with exposure and bioactivity estimates from ExpoCast and Tox21, respectively. Chemicals with elevated exposure and/or toxicity potential were further examined using a mixture of 100 chemical standards. A total of 33 chemicals were confirmed present in the dust samples by formula and retention time match; over half of these have not been associated with dust in published literature. Chemical matches found in at least 10 of the 56 dust samples include Piperine, N,N-Diethyl-m-toluamide (DEET), Triclocarban, Diethyl phthalate (DEP), Propylparaben, Methylparaben, and Tris(1,3-dichloro-2-propyl)phosphate (TDCPP). This study demonstrates a novel suspect screening methodology to prioritize emerging contaminants for subsequent targeted analysis. The methods described here rely on strategic integration of available public resources and should be considered in future non-targeted and suspect screening assessments of environmental and biological media.

3540 Considering Possible Mechanisms of Toxicity for Phthalates on California's Proposition 65 List Using Bioseek Data from ToxCast

N. T. Pham[1], S. Iyer[1], M. Marty[1], M. Sandy[1], G. Solomon[2] and L. Zeise[1]. [1]*Office of Environmental Health and Hazard Assessment, California EPA, Sacramento, CA and* [2]*Office of Secretary, California EPA, Sacramento, CA.*

Many chemicals in commerce have poorly characterized modes of action. *In vitro* high-throughput screening (HTS) methods are an approach to assess chemical effects across multiple cellular pathways. The present study uses the HTS data available from the US Environmental Protection Agency's Toxicity Forecaster (ToxCast™) repository to attempt to better understand the mode of action of selected phthalates. We selected the six phthalates listed under California's Proposition 65, which requires the State to maintain and update a list of chemicals "known by the State" to cause cancer or reproductive toxicity. These are butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), dihexyl phthalate (DHP), diisodecyl phthalate (DIDP), and diisononyl phthalate (DINP). We evaluated the activity of these phthalates in the BioSeek (BSK) platform in ToxCast and used the Toxicological Priority Index (ToxPi) software as a visualization method to assess the chemical activities in these assays in relation to the known toxicological endpoints of these phthalates. DINP was not tested in the BSK platform. DIDP did not activate any BSK assays. The remaining four phthalates activated several of the same assays (e.g. BSK_4H_MCP1_down, BSK_SAg_CD40_down), which may indicate similar modes of action among the chemicals. Chemical-specific activity in target assays was also noted across the assay platform surveyed. For instance, DEHP was the only phthalate to activate assays associated with interleukin 6 (IL6) and tumor necrosis factor α (TNFα). This may indicate that certain phthalates are active in a wider range of biological pathways than others, which may result in additional toxicities. These initial findings demonstrate the potential use of ToxCast to assess modes of action in connection to downstream toxicological endpoints. Such an application may assist in evaluating chemicals in commerce for testing prioritization.

3541 Toxicant Bioavailability When Using Low Volume Polymer Based Organ-on-a-Chip Bioreactors to Study Exposures to Chemical Contaminants

D. A. Markov, P. Fryman and L. J. McCawley. *Biomedical Engineering, Vanderbilt University, Nashville, TN.* Sponsor: L. Vernetti.

Recent advances and successes in microfabricated organs-on-chips and human organ constructs have made it possible to design scaled and interconnected organ systems that may significantly augment the current development of new model systems for toxicant assessment and for monitoring dynamic toxicant-dependent changes to cellular functions and signaling cascades. Our group is developing a microfabricated Mammary Gland Thick Tissue Bioreactor (MG-TTB) to support the combination of 3D culture methods with controlled chemical exposure to investigate potential mammary gland toxicity. We utilize polydimethylsiloxane (PDMS), a biologically inert and commonly used material in organ-on-a-chip devices. One concern in using PDMS for cell culture and drug-testing platform applications is the potential loss of molecules, drugs, nutrients or toxicants from the media into the substrate that could result in an erroneous estimation of dose response curves and lowest-observed-adverse-effect-level (LOAEL). We have tested adsorption to PDMS surface a variety of toxicants with Log P values ranging from -2.93 to 7 that are currently being used in MG-TTB model validation. These include compounds with known adverse effects on mammy development *in vivo* (ie., Diethylstilbestrol, Estradiol benzoate, Tamoxifen, Secoisolariciresinol diglucoside, Genistein, Bisphenol A, Nonylphenol, Dioxin, Polybrominated diphenyl ethers), as well as control compounds with no effect (ie., Aminopyralid, Foramsulfuron, Imazaquin). We have found that PDMS treatments (Oxidized vs Non-oxidized, high temperature Annealed vs Non-Annealed, and stiff (10:2) formulation vs regular (10:1) formulation) did not have appreciable effect on toxicant interaction with the bioreactor surface for all of Log P values. We have also found that there was less than 5% loss for compounds with Log P value of 3.4 (Bis-phenol A) or less. We believe our findings will be useful for larger community by evaluating how toxins interact with the surfaces of materials commonly used in organ-on-a-chip microfluidic device fabrication.

3542 Exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Affects Vascular Development in the Zebrafish Eye, Gut, and Trunk

M. S. Yue[2], M. R. Taylor[2], R. E. Peterson[2] and J. S. Plavicki[2]. [1]*Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI and* [2]*School of Pharmacy, University of Wisconsin, Madison, WI.* Sponsor: R. Peterson.

TCDD, a ubiquitous environmental contaminant, causes developmental toxicity by activating the aryl hydrocarbon receptor (AHR) pathway. In zebrafish, embryonic exposure to TCDD causes cardiovascular dysfunction, hemorrhaging, pericardial edema, and many other effects, eventually culminating in morbidity. Studies in mouse, chick, and fish models indicate that the AHR pathway plays an important role in the resolution of vascular structures during early development, highlighting the vasculature as a potential target for TCDD-induced toxicity. Here, we investigated the effects of TCDD exposure (1 ng/mL, waterborne) on vascular development in the eye, presumptive digestive system, and trunk of the zebrafish embryo. We utilized high-resolution live imaging and video microscopy, combined with transgenic fish with fluorescent markers for endothelial cells (kdrl:EGFP) and erythrocytes (gata1:DsRed) to gain insight into how TCDD affects the vascular architecture and regional blood flow over time. TCDD reduces heart rate and cardiac output beginning at 72 hours post fertilization (hpf). Consequently, we are imaging embryos at 30, 48, and 60 hpf. Our results indicate that TCDD exposure results in previously unreported vascular phenotypes in the retina, gut, and trunk. To further understand how TCDD-induced activation of AHR in the vascular endothelium contributes to the observed TCDD phenotypes, we are generating transgenic fish lines to express constitutively activated AHR (caAHR) specifically in endothelial cells. We are utilizing the Gal4/UAS and Tet-On systems to ensure consistent and uniform caAHR expression throughout the vasculature. In addition, the Tet-On system allows for temporal control of endothelial-specific caAHR expression, enabling us to activate expression of caAHR signaling at particular stages of vascular development and in adult fish. (Supported by NIEHS K99ES023848 and UW-Madison Wisconsin Alumni Research Foundation)

3543 Metabolic Competent Expandable Human Upcyte Hepatocytes Enable Metabolism Studies Including CYP2D6 Dependent Pathways

A. Noerenberg[2], W. Schroth[1], M. Thomas[1], T. Mürdter[1], J. Johänning[1] and S. Heinz[2]. [1]*Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology and University of Tuebingen, Stuttgart, Germany and* [2]*upcyte technologies GmbH, Hamburg, Germany.* Sponsor: P. Hewitt.

Primary human hepatocytes have limitations for drug testing due to the low availability of human liver tissue. To address this, we have improved our upcyte technology enabling primary human hepatocytes to proliferate up to 40 doublings whilst still retaining a highly metabolic competent phenotype when cultured at confluence. The resulting cells are called "upcyte hepatocytes" and enable studies of metabolic clearance, hepatotoxicity and drug-drug interactions. Here, we tested upcyte hepatocytes from donor 653-03 and its genetically modified, CYP2D6 expressing derivative (653-03-2D6) for their enzymatic activities of CYP1A2, 2C8, 2B6, 2C19, 2D6, 2C9, 3A4 using a substrate cocktail incubation and Triple Quad MS analysis. In addition 75 ADME related genes were quantified using Fluidigm Taqman array (Biomark). upcyte hepatocytes show high basal activities of phase I enzymes which are inducible in the range of 2 to 23 fold by prototypical inducers rifampicin (CYP3A4, CYP2C8, CYP2C9, CYP2C19) and phenobarbital (CYP2B6) indicating functional PXR/CAR mediated CYP induction. On the level of gene expression, induction of CYP gene expression followed the same pattern as observed based on activities. Overall, there was no difference in the global regulatory gene expression pattern comparing both cell strains. upcyte hepatocytes from donor 653-03 express a number of endogenous CYP enzymes yet show low levels (5 pmol/mL) of CYP2D6 activity. In the 653-03-2D6 cell strain, CYP2D6 is stably expressed with a basal activity of over 1600 pmol/mL. In comparison, HepaRG cells almost completely lack CYP2D6 activity with less than 1 pmol/mL. In addition, this study shows that the recombinant expression of CYP2D6 does not largely influence other CYP activities, thus enabling complex metabolic profile analysis of CYP2D6 dependent drugs such as the breast cancer antiestrogen tamoxifen. In conclusion, our data show that upcyte hepatocytes due to their possibility of expansion (up to 12x10^9 cells per donor) combined with long-term flexible expression of adult hepatic phenotypes constitute novel *in vitro* liver cell models for CYP2D6-dependent and independent metabolism and toxicity analysis in pharmaceutical drug development.

3544 Toxicity Assessment of Co-Culture Human Systems During Serial Multi-Well Gradient Exposures

M. B. McCartney[1], D. J. Sloan[1], J. Gordon[2] and R. McClelland[1]. [1]*SciKon Innovation, Chapel Hill, NC and* [2]*The Tauri Group, Frederick, MD.*

We evaluate the robustness and applicability of a newly designed microfluidic systems to mimic passage of biological fluids and materials from one tissue type to another within a 96-well tissue culture system. A goal for applying this technology is to couple, in series, intestine and hepatocyte cell models through microfluidics for a complete benchtop first pass metabolic and toxicity system. Uptake of drugs into the intestinal epithelium and subsequent metabolism prior to entering the enterohepatic circulation influences the bioavailability of drugs and chemicals. Failure to account for the first pass effects of the intestine can lead to unwelcome surprises of reduced efficacy and unwanted toxicity during expensive first in human testing. The inadequacy of animal models to predict human biology in the drug development process is becoming increasingly clear due to species differences in uptake and metabolism at both the cellular and organ levels. As a result, there is a need for more human model systems to be incorporated earlier in research and development. Innovative concepts such as "body-on-a-chip" have been introduced, but the complexity and miniaturization of the many of the formats has limited applicability on a commercial scale. SciKon is developing tools that better recapitulate biological systems. We show here the evaluation of a fluidics system engineered to connect 10-wells of each row in a 96-well plate together with a microfluidic channel. This system sequentially links wells together to form a cascade of cell chambers through which drugs or toxicants can be applied. Toxicants interact with cells in the upstream compartments (e.g. intestinal) creating metabolites that will mix and interact in downstream wells (e.g. hepatic) forming a parent-metabolite gradient in a time-resolved and gradient fashion. Such a system enables concentration by time kinetics of toxicity measurements in a more life-like environment. We have evaluated the effects of acetaminophen on primary human intestinal and hepatic cells in comparison to static conditions using the reagent CellTox Green. Using a fluorescein tracer molecule we demonstrate that exposures in the fluidic system were non-linear and shaped similarly to an expected plasma curve *in vivo*. Comparisons of area-under-the-curve (AUC) between static and flow systems revealed dramatically different dose-response curve shapes and EC-50s.

3545 Quantitative, Label-Free Real-Time Kinetic Monitoring of the Effects of Toxic Compounds in Live Cells by Holographic Imaging Cytometry

K. Alm[1], B. Janicke[1], E. Luther[3], M. Sebesta[1], P. Egelberg[1] and E. Holden[2]. [1]*Phase Holographic Imaging AB, Lund, Sweden;* [2]*SeeCyte Biosciences, Boston, MA and* [3]*Department of Pharmaceutical Sciences, Northeastern University, Boston, MA.* Sponsor: P. Narayanan.

The use of live cellular assays is widely applied to the assessment of drug toxicity and safety testing of household chemicals, cosmetics, tobacco products and various environmental pollutants. The majority of cellular assays require toxic stains which hinder the physiological relevancy and often alter the intactness of the investigational models. We developed and validated a non-invasive label-free imaging cytometry platform Holomonitor®M4 (Phase Holographic Imaging, Lund, Sweden), and applied it to the assessment of drug effects on various cell lines. HoloMonitor M4 enables quantitative, label-free analysis of living cells over long periods of time while operating in an incubator. A low-power red laser creates an interference pattern (hologram) which is reconstructed by software into images. Automated software algorithms are applied for cellular segmentation, cell tracking, calculating quantitative features (optical thickness and volume), and a wealth of morphological parameters (i.e. area and texture amongst others). Additionally, we export 2-dimensional images for each time point into Image J. This allows us to produce 4-dimensional plots of cellular behavior over time: X position, Y position, cell thickness in gray scale coded as brightness and elapsed time in the Z-direction. In our experiments we used HeLa, SKOV3, L929 and HT1080 cells which were cultured in 35 mm Petri dishes, 6- and 96-well microtiter plates and imaged in 5 minute intervals for 2 to 3 days untreated or after application of cytotoxic drugs Etoposide, Colchicine, Taxol and Doxorubicin. We established visual and quantitative baselines in untreated cell lines for cell cycle and proliferation. We were able to determine effects of pharmaceutical compounds on the cell cycle, and distinguish compound-specific modes of cell death via mitotic dysfunction and catastrophe, apoptosis and necrosis. Label-free, quantitative real-time monitoring of cellular processes offers

distinct and important advantages with the possibility of evaluating effects of pharmacological compounds with more physiological relevancy and less experimental complexity. Continuous real-time monitoring allows characterization of the time course of the compounds effects for several cellular generations.

3546 Evaluation of the Performance of the Keratinosens™ Assay with Added Metabolic Capability

R. Settivari[2], N. Visconti[2], R. Acosta Amado[1] and S. Gehen[1]. [1]*Dow AgroSciences, Indianapolis, IN and* [2]*Dow Chemical, Midland, MI.*

The KeratinoSens™ assay has emerged as one of the most promising *in vitro* assays for predicting skin sensitization to chemicals. OECD guideline 442D for the KeratinoSens™ assay states that a positive result in the assay is conclusive, whereas, a negative result is considered as inconclusive. Since some chemicals become sensitizers only after being metabolized (prohaptens), false negative predictions for such compounds in the KeratinoSens™ assay may be due to a lack of sufficient metabolic capability of the keratinocytes used in the assay. In the present study, the performance of the KeratinoSens™ assay with added metabolic capability (microsomes or S9 fractions) was evaluated on a set of 4 prohaptens, 5 sensitizers, and 3 non-sensitizers as determined by prior *in vivo* and *in vitro* studies. Initially, excess cytotoxicity and bacterial contamination was observed in KeratinoSens™ cells cultured for 48 h with microsomes. As a result, subsequent studies focused on using S9 fractions from rat liver, rat skin, human liver and human skin. For rat hepatic S9, the optimal concentration was determined to be 1% (v/v) based on its metabolic capacity and associated cytotoxicity. The same concentration of S9 fraction was used for all S9 types. The S9 fractions did not impact results for sensitizers and non-sensitizers as the KeratinoSens™ assay correctly predicted all tested sensitizers and non-sensitizers in both with and without S9 incubation experiments. In the absence of S9 incubation, three of the four tested prohaptens were negative in the KeratinoSens™ assay. All prohaptens were predicted correctly in KeratinoSens™ assays with added rat or human hepatic S9, whereas when skin S9 was used, three out of four prohaptens were correctly predicted *in vitro*. Overall, these observations indicate that the addition of S9 to the KeratinoSens™assay improved the accuracy for predicting prohaptens and non-sensitizers. The addition of metabolic capability to the KeratinoSens™ assay therefore enhances confidence in the accuracy of true negative results.

3547 Skin Irritation of Medical Devices: *In Vitro* Assay with Episkin Reconstructed Human Epidermis (RHE)

C. Pellevoisin[2], C. Tornier[2], C. Gregoire[2], B. Rollins[1], D. Briotet[3], A. Turley[4], M. Lee[4], Y. Haishima[5] and N. Seyler[2]. [1]*Arthrex Laboratory, Naples, FL;* [2]*EPISKIN Academy, Lyon, France;* [3]*Namsa, Chasse sur Rhone, France;* [4]*Nelson Laboratories, Salt Lake City, UT and* [5]*NIHS, Tokyo, Japan.* Sponsor: E. DuFour.

Skin irritation is one the 3 toxicological endpoints that are always assessed for medical devices. Yet, even if the ISO 10993-10 norm mentions the *in vitro* OECD test guideline (TG439) for *in vitro* skin irritation of chemical, the reference test method remains the animal one. The aim of this work is to develop an *in vitro* protocol to assess the potential of medical devices and their constituent materials to produce skin irritation. The protocol is an adaptation of the TG 439 based on the reconstructed human epidermis Episkin RHE. This method is validated for neat chemicals but not for medical device extracts (ISO 10993-10) in which potential leached irritants are diluted in a solvent. The original protocol has been adapted to meet these specificities by increasing the volume of the product tested (100microl), the time of exposure (24h) and by suppressing the post-incubation step. The protocol have been challenged with different irritant chemicals; Sodium Dodecyl Sulfate (SDS), Lauryl Acetate (LA) and Heptanoic Acid (HA) diluted in two solvents, one polar, phosphate buffer saline (PBS) and one apolar Sesame oil (SO) at final concentrations of 4%, 2%, 1% and 0.1%. Cell viability was measured with MTT assay, IL-1α release quantified in the culture medium by ELISA and histological analysis of the tissues performed. The second part, of the work present the results of the assay performed on different polymers used in medical devices industry, polyvinyl chloride (PVC) sheets spiked with Genapol X-080 a non ionic detergent, and Silicone spiked with SDS an ionic detergent. The polymers are prepared according to the norm ISO10993-12. 60 cm2 are cut in small pieces and extracted in 20 ml of polar (PBS) and apolar (SO) solvents at 50°C for 72 ±2h under agitation. The assay is conducted the day of the end of the extraction procedure. The results show that this *in vitro* protocol developed with the Episkin RHE model allows to detect low concentration of irritant spiked in polar and apolar solvents. Likewise, this assay is able to detect in the extraction solvent of several polymers the presence of leached irritants compared to control without irritant. These polymers are candidates to be used as extractable positive controls for *in vitro* skin irritation testing of medical devices.

3548 Development of an Experimental Cell-Based Approach to Evaluate Biological Effects of Aerosols from the Application of Hair-Straightening Products *In Vitro* Using Relevant Product Application and Cell Exposure Conditions

D. Ritter[1], A. Bitsch[1], M. Elend[1], S. Schuchardt[1], T. Hansen[1], C. Brodbeck[2], J. Knebel[1], R. Fautz[3], A. Fuchs[3] and C. Gronewold[3]. [1]*Fraunhofer ITEM, Hannover, Germany;* [2]*Fraunhofer SCAI, Sankt Augustin, Germany and* [3]*Safety and Toxicology, KAO Germany GmbH, Darmstadt, Germany.* Sponsor: C. Dasenbrock.

Aerosols are generated during the application of hair-straightening products by professional hairdressers. The aerosols include a considerable amount of inhalable particles. Occupational safety assessment for the gaseous phase can be conducted based on known constituents, which are toxicologically characterized such as aldehydes, acetone and siloxanes. However, this is not possible for the particulate phase of the aerosol, since composition and combinatory biological effects of the native aerosol mixtures compounds are largely unknown. An experimental setup was established with real hair strands and straightening product usage under consumer relevant conditions. It included an optimized box for product application and an exposure device for air-lifted interface exposure of cells (P.R.I.T.® AE-Box and ExpoCube®) as well as online particle monitoring (GRIMM Mini-WRAS 1371). Additional chemical analysis was carried out offline by HPLC. Product application on hair strands led to a reproducible aerosol generation. Human lung cells (A549) were exposed in an acute toxicity design (viability and IL-8 secretion). Reliability and relevance of the experimental approach were controlled by empty-box exposures and controls such as lactose and cupper-II-sulfate particles sharing particle size ranges and concentrations comparable to the aerosol from hair treatments. In a first set of experiments, exposures to positive controls induced loss of cellular viability and induction of IL-8 secretion. Cellular changes after exposure to negative controls or aerosols from hair treatments were comparable. In conclusion, this experimental approach seems to be a valuable way to test biological effects of aerosols from consumer relevant product applications, since it offers the possibility to study varying biological effects under highly relevant exposure conditions.

3549 *In Vitro* Prediction of Antiretroviral Drug-Induced Hepatotoxicity by Using Mitochondrial MiToxView® Screening Platform

M. Porceddu[2], C. Pertuiset[2], S. Camus[1], C. Chesné[1], N. Buron[2] and A. Borgne-Sanchez[2]. [1]*Biopredic International, Saint Grégoire, France and* [2]*Mitologics, Romainville, France.* Sponsor: N. Claude.

Drug-induced mitochondrial dysfunction in liver can cause microvesicular steatosis, steatohepatitis and cytolytic hepatitis. Liver susceptibility is even more increased in HIV, HBV and HCV infected patients explaining acute hepatotoxicity of several antiretroviral drugs, occurring during clinical trials but not predicted by preclinical studies. To improve early prediction of DILI in human, we developed MiToxView®, a medium-throughput *in vitro* screening platform to detect loss of mitochondrial function and integrity by using isolated mouse liver mitochondria [1]. These assays have been transposed to mitochondria purified from the human hepatic cell line HepaRG®, which presents mitochondrial activity profile closed to human primary hepatocytes. Mitochondrial toxicity of 2 protease inhibitors (ritonavir and saquinavir) and 3 nucleoside analogs (fialuridine, zalcitabine and zidovudine) have been investigated in HepaRG model. Acute mitochondrial toxicity was explored on isolated HepaRG mitochondria by measuring oxygen consumption, swelling and transmembrane potential while long-term and/or metabolite-induced mitochondrial toxicity was investigated in cultured differentiated HepaRG cells by measuring cell viability, oxygen consumption, mitochondrial transmembrane potential and mtDNA content. Results showed that protease inhibitors presented direct acute mitochondrial toxicity while nucleoside analogs only induced long-term mitochondrial toxicity, with all tested drugs leading to mitochondrial respiratory chain dysfunction. This combination of assays on HepaRG mitochondria and cultured cells represents a valuable model to detect hepatotoxicity potential of antiretroviral compounds.[1] Porceddu M, Buron N, Roussel C, Labbe G, Fromenty B, Borgne-Sanchez A. (2012). Toxicol Sci. 129: 332-45.

3550 Classifying Modes of Action of Electronic Cigarette Toxicants in Differentiated Cells and Stem Cells Using a Multiplexing Video Bioinformatics Platform

A. Zahedi[3], R. Phandthong[3], V. On[3] and P. Talbot[3]. [1]*Bioengineering, University of California Riverside, Riverside, CA;* [2]*Cell Biology & Neuroscience, University of California Riverside, Riverside, CA and* [3]*Electrical Engineering, University of California Riverside, Riverside, CA.*

Toxicant potency and type of stress responses are dependent on specific cell types. However, biochemical and toxicological screening of multiple cell types is costly, tedious, and requires a large supply of biological samples. We have developed a high-throughput cytotoxicity platform to screen and identify multiple modes of action in differentiated cells and stem cells. A video bioinformatics approach was used to analyze time-lapse phase contrast and fluorescent videos of live cells and extract biomarkers of cell harm. The system was tested on pulmonary fibroblasts (PFs), alveolar lung epithelial cells (A549), and neural stem cells (NSCs). Cells were exposed to residue from electronic cigarettes (ECs), popular devices that are marketed as harm reduction or smoking cessation alternatives. These devices have been shown to release harmful chemicals and by-products such as metal nanoparticles. During treatment with EC aerosols and liquids, cells were placed in a BioStationCT high-content incubation/imaging chamber, and high-resolution videos were collected. Videos were analyzed using customizable algorithms developed in CellQuant, Matlab, and CellProfiler software. These algorithms were used to quantify treatment-induced changes in morphology, cell growth, cell division, motility, and apoptosis, as well as morphological and dynamic dysfunctions/irregularities in intracellular organelles including vacuoles, autophagosomes and mitochondria. EC treatment produced different effects in different cell types such as: 1) cytoskeletal fragmentation and apoptosis in PFs, 2) inhibition of cytokinesis and accumulation of vacuoles/autophagosomes in A549s, and 3) altered mitochondrial and autophagy dynamics in NSCs. This approach allows biologists without training in computer programming to automatically identify and quantify multiple modes of action from large data sets. It also enables scientists to design and prioritize experimental paradigms for further study of underlying pathologies.

3551 Surface Plasmon Resonance Microarray-Based Functional Phenotyping to Measure Toxicant Effects on Hematopoietic Stem Cells

M. A. Lynes[3], D. A. Knecht[3], E. F. Guignon[1] and D. A. Lawrence[2]. [1]*Ciencia, Inc., East Hartford, CT;* [2]*Immunology, Wadsworth Center, Albany, NY and* [3]*Molecular and Cell Biology, University of Connecticut, Storrs, CT.*

Surface plasmon resonance (SPR) is a phenomenon that has been widely used to measure molecular interactions at a metal:dielectric interface. Traditional measurements using this phenomenon employ the Kretschmann optical configuration (e.g. prism coupled SPR) to match the phase velocity of incident light with the resonance requirements of the plasmon. We have engineered a grating-coupled SPR instrument that eliminates the need for a prism to accomplish this resonance, and have shown in past work that this instrument can be used for the assessment of molecular interactions, as well as measurement of viral particles, bacteria and mammalian cells in a microarray format. At present, we are adapting this instrument platform to take advantage of its sensitivity in a small sample volume interrogation of the functional capabilities of stem cells captured on a spatially encoded microarray. The consequences of exposure of the captured cells to a toxicant gradient (e.g., CdCl2) formed in a low melting point agarose overlay can be assessed by the progressive expression of differentiation surface markers and the secretion of cytokines using SPR-coupled emissions as indicators of the cellular developmental stage. In preliminary studies, we have used flow cytometry to assess the effect of 20 µM CdCl2 on cell lines representing different stages of B cell differentiation and have measured stage-specific sensitivity to this toxicant. The level of the stress response protein metallothionein (MT) is also different between these cell lines. Since exposure of spatially encoded cells to an overlaying gradient will dramatically reduce reagent and material costs while simultaneously reducing labor costs, we anticipate that such an assay will dramatically enhance our ability to assess the developmental consequences of toxicant exposure in near real time.

3552 Evaluation of the Stability of Reconstituted Human Bronchial Epithelium, MucilAir, During 4 Weeks in Culture

S. Constant[2], S. Frentzel[3], M. Gaca[1], D. Grandolfo[3], P. Leroy[3], L. E. Haswell[1], J. Hoeng[3], S. Huang[2], K. Luettich[3], E. Minet[1], M. Monachino[2], K. Trivedi[3] and L. Wiszniewski[2]. [1]*British American Tobacco R&D, Southampton, United Kingdom;* [2]*Epithelix, Plan-les-Ouates, Switzerland and* [3]*Philip Morris International R&D, Neuchatel, Switzerland.*

MucilAir, a 3D cell model of the human airway epithelium, could be a valuable *in vitro* tool for studying respiratory diseases and assessing inhaled toxicants in long-term or repeated dose toxicity testing. Therefore it is important to assess the stability of tissue morphology and function over time. This study assessed the stability of bronchial MucilAir tissues in three independent laboratories over 38 days of culture post-differentiation. The endpoints tested were transepithelial electrical resistance (TEER), cilia beat frequency (CBF) and mucin secretion, conducted simultaneously at all three test sites. TEER values were comparable and proved to be relatively stable over the culture period, despite one site having a slight increase in TEER after 14 days in culture. Similarly, CBF did not change markedly over time. However, the average CBF varied across the three sites, ranging from 9.73±0.16 to 12.26±0.15 Hz. Cilia active area measures were somewhat variable within day, but similar between two of the three test sites, with the third reporting generally lower percentages. A similar observation was made for mucin production with comparable values in two of the three test sites. However, the results obtained at the third test site were generally higher although reporting stable mucin production over the 38 days. Despite some minor differences between the test sites, the overall results demonstrated that the fully differentiated bronchial MucilAir tissue was stable over a 38 day culture at three independent laboratories. To support these initial findings, immunohistological analysis for specific cellular markers (p63, MUC5AC, FoxJ1, Ki67) is being employed to evaluate the frequency and distribution of cell types within the cultures before embarking on future studies using various test materials.

3553 Combining a Real-Time *In Vitro* Cell Viability Assay and RNA Extraction from the Same 3D Spheroids

T. Riss, S. Duellman, B. Hook, M. Bratz and S. Lewis. *Promega Corporation, Madison, WI.*

The use of three dimensional cell culture is proving to provide more physiologically relevant models for studying *in vitro* toxicity. Although there is wide implementation of these models, there is an unmet need to establish *in vitro* assay methods to interrogate larger and more complex 3D structures. The rationale of this work was to determine if high quality RNA could be extracted from 3D spheroids following cell viability measurements using a new real-time assay chemistry. HEK293 cell spheroids were generated using the hanging drop method and cultured in the presence or absence of the cell viability reagent to determine if it had any effect on the ability to extract RNA. Cell viability measurements were performed by monitoring generation of a luminogenic substrate. RNA was extracted from individual spheroids using either a manual or an automated method. RNA yield was determined using a fluorescent RNA-binding dye and RNA integrity (RIN value) was determined using a bioanalyzer. The results showed the % CV for the cell viability measurements from 24 samples of spheroids was from 9.0-10.2, demonstrating the hanging drop method is highly reproducible for generating 3D cultures. The average yield of RNA extracted from 440 um spheroids was 77 ng in the presence and 74 ng in the absence of the real-time viability detection reagent. The RIN values were all above 0.8 indicating excellent quality of RNA using the manual and automated methods. A comparison of the Ct values generated from RNA isolated from 4 different cell types showed the presence of the real-time assay reagent did not have an effect. We conclude the real-time cell viability assay chemistry is compatible with downstream multiplexing of RNA extraction for subsequent analysis of gene expression. The results of RNA extraction from the same samples used for viability measurements demonstrate the presence of the real-time cell viability reagent did not have an effect on yield or integrity of the RNA. Multiplexing provides a more efficient approach compared to evaluating replicate samples from parallel assay plates.

3554 High Throughput 3D Spheroid Culture of Corning® HepatoCells and Liver Toxicity Applications

F. Li[1], R. Zuo[1], M. Andes[1], S. Parikh[1], K. Cooper[1], R. A. Faris[2] and C. J. Patten[1]. [1]*Corning Incorporated-Life Sciences, Bedford, MA and* [2]*Corning Incorporated-Life Sciences, Corning, NY.* Sponsor: C. Crespi.

In vitro hepatic 3D cultures, based on single cell line or mixed cell types, have been shown to better reflect the *in vivo* behaviors of liver cells including proper polarization, cell-cell contacts and hepatic cellular functions. These advanced systems are currently being evaluated by both industrial and academic scientists for drug discovery and drug development research. Corning® HepatoCells is a renewable hepatic cell source derived from primary human hepatocytes with consistent phenotypes for cytochromes P450 enzyme and drug transporter activities, which are necessary for ADME applications such as CYP induction, metabolism and cytotoxicity studies. Here, we report the adaptation of Corning® HepatoCells to *in vitro* 3D culture using Corning® ULA Spheroid microplates (96-well or 384-well format). Spheroids made from Corning® HepatoCells are uniform in size, shape, and consistent in measurement of ATP levels. Day 4 or day 7 single spheroids made from 1,500 HepatoCells were treated for 24 hours with known liver toxins such as aflatoxin B1, acetaminophen and diclofenac. Bioluminescent ATP assays were performed for viability endpoint measurement. Our data indicate that 3D spheroid cultures significantly improve the drug metabolism activity (i.e. CYP3A4) and cytotoxicity responses in comparison to 2D cultures. Importantly, the dose response EC50s of known liver toxins from HepatoCells spheroid tests are consistent with other *in vitro* models in comparison. Together, our results showcase a promising 3D spheroid culture based platform using Corning® HepatoCells and Corning® ULA Spheroid microplates for high throughput screening of liver toxins.

3555 3D Cell Clusters for Predictive Toxicity Analysis

L. Stehno-Bittel, S. J. Williams and K. Ramachandran. *Likarda, LLC, Kansas City, KS.* Sponsor: M. Czerwinski.

The ability of *in vitro* assays to produce reliable and medically-relevant information is essential to new drug discovery. Cell culture lines grown in monolayers are the standard tool for toxicity screens, yet there are significant limitations with this model. Namely the results often do not predict what will happen in the body. 3D cell culture has quickly become the new standard for drug screening, especially in the cancer field, yet it has been slow to take hold for *in vitro* toxicology testing. Comparing the same cell types grown in 2D versus 3D, researchers have shown differences in the protein profiles, gene expression, signal transduction, cellular growth, function and morphology. We have published studies with known anti-cancer and anti-diabetes drugs on cells cultured in 3D showing that they were more predictive of the known *in vivo* response than the 2D outcomes. The goal of this study was to optimize the use of 3D hepatocyte cell clusters to standard *in vitro* toxicology assays and determine their predictive abilities. Cells of the transformed hepatic cell line (HepaRG) were tested in standard 2D monolayers and in 3D cell clusters. HepaRG clusters were formed by loading the cells into glass micromolds that resulted in formation of 3D cluster in 2 days. Clusters were washed from the molds and measured for size. The average diameter was between 75–100 µm. We have shown from previous work that cell clusters with a diameter over 125 µm have significant diffusion limitations that reduce the exposure of the core cells to test products. Viability assays of the 3D HepaRG clusters on days 5-10 showed high viability with 99% live cells. Cytochrome P450 (as measured by P450-Glo, Promega)) was 20% greater in the 3D cell clusters than the 2D monolayer, but when normalized to cell number, the 3D clusters had a CYP450 luminescence value that was 100 times greater than the same cells cultured in 2D. One problem is that the 3D cell clusters tend to stick to surfaces and new culture techniques are being developed to optimize the metabolism assays in 3D.

3556 Substrate-Independent Real-Time Toxicity Screening in 3D Human Tissue Culture Models

T. Xu[1,2], H. Lam[2], D. Close[1], G. Sayler[1,2] and S. Ripp[1,2]. [1]*490 BioTech Inc., Knoxville, TN and* [2]*Center for Environmental Biotechnology, The University of Tennessee, Knoxville, TN.*

To assuage public health concerns resulting from the vast catalog of compounds that are used for developing economical and robust consumer products, *in vitro* toxicity assays are now a requisite step for compound safety evaluation. However, current cell-based toxicity assays have not evolved to support high throughput screening of this chemical backlog under physiologically relevant conditions due to their reliance on chemical or photonic stimuli to generate their signals. The inherent limitations of photobleaching/phototoxicity from repeated photostimulation and/or cellular destruction concurrent with chemical luciferin application restricts assay throughput by providing only intermittent output signals, while their requisite stimulation prevents application under modern 3D conditions that more closely mimic the cell's natural *in vivo* environment. To circumvent these limitations, we have a developed a synthetic luciferase that can be integrated into human cells to self-generate a bioluminescent signal representative of host metabolic activity level dynamics without any external stimulation. This approach permits continuous or repeated monitoring of samples under 2D and 3D culture conditions without exogenous interaction. To investigate the response of autobioluminescent cells to toxicological challenge under 2D and 3D growth conditions, autobioluminescent human embryonic kidney (HEK293) cells capable of self-modulating their autobioluminescent signal in response to metabolic activity were seeded as a monolayer or encapsulated in collagen hydrogel, and exposed to the known metabolic inhibitor, Zeocin. Significant decreases in bioluminescence were observed after 48 h of exposure to 0.4 and 1.0 mg/ml Zeocin under 2D and 3D conditions, respectively. HEK293 cells programmed to auto-initiate expression of the synthetic luciferase using a Tet-On promoter responded to doxycycline stimulation in a similar time-dependent manner under 2D and 3D conditions, however, while 2D-grown cells produced a 16-fold increase, encapsulated cells demonstrated a 6-fold induction. These data suggest that traditional 2D monolayer-based assays overestimate toxicity relative to assays employing 3D growth techniques, and that the use of a fully autonomous reporter can allow these more physiologically relevant assays to be performed under high throughput conditions.

3557 Hepatocyte Spheroid Cultures in Galactosylated Cellulosic Sponge for Drug DMPK and Efficacy Testing

A. Ananthanarayanan[2], Y. Qu[2], N. Hari Singh[2], B. Nugraha[1], M. McMillian[2] and H. Yu[3]. [1]*Imperial college London, London, United Kingdom, Singapore, Singapore;* [2]*Invitrocue Pte Ltd, Singapore, Singapore and* [3]*Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.* Sponsor: M. Johnson.

Hepatocyte spheroids are gaining increasing acceptance for various drug discovery applications. Formation of multiple spheroids per well with uniform dimensions has been challenging. We have developed a galactosylated cellulosic scaffold with uniform macroporosity (Nugraha et al, 2011), allowing culture of hepatocytes from rat, human and monkey hepatocytes of uniform spheroid size of between 80 µm–120 µm. The hepatocyte spheroids exhibit enhanced polarity with localization of MRP 2 and CD147 proteins similar to that observed *in vivo*. The spheroids also maintain their viability over four weeks in culture and maintain mature hepatocyte specific functions over 2 weeks in culture. The cells are inducible for all major CYP 450 enzymes with responses higher than that observed in sandwich cultured hepatocytes. The platform has been characterized for infectious disease applications. Owing to appropriate formation and maintenance of polarity the spheroid cultured cells allow for increased HCV uptake and maintain similar level of uptake over 14 days. The spheroids also allow for 8 fold increase in viral titers compared to monolayer cultures (Ananthanarayanan et al, 2014). We have also modeled the liver stage of *P. cynamolgi*. The spheroids are infected at higher levels than monolayer cultures and allow for proliferation of parasite *in vitro*. We are able to recapitulate the entire lifecycle of *P. cynamolgi in vitro*, wherein we are able to recapitulate the liver stage and *P. cynamolgi* release and reinfection in erythrocytes. 3D spheroid cultures using galactosylated cellulosic sponge offer advantages for screening novel candidates for drug DMPK and efficacy against pathogens.

3558 Validation and Assessment of miRNA122 as a Clinical Biomarker of Hepatotoxicity Utilizing Affymetrix QuantiGene Technology

D. Burt[2], S. Schomaker[2], R. Warner[1], K. Johnson[1] and J. Aubrecht[2].
[1]Dept. of Pathology, University of Michigan Medical School, Ann Arbor, MI and [2]Drug Safety R&D, Pfizer Inc., Groton, CT.

Hepatotoxicity is major challenge in drug development. While alanine aminotransferase (ALT) remains the gold standard biomarker of liver injury, miR122 has been proposed as an alternative biomarker that has a potential to better predict the potential for drug induced liver injury and/or address its mechanism. To facilitate the application of miR122 in a clinical setting where PCR-based methods are not readily available, we validated the Affymetrix QuantiGene miRNA122 assay and evaluated its utility for analysis of serum samples from human subjects with a variety of liver impairments. The Affymetrix QuanitiGene platform is a 96-well plate based-sandwich nucleic acid hybridization technology with signal amplification via a branched DNA amplifier and chemiluminescence signal generation. This technology eliminates the need for reverse transcription, specialized instrumentation and allows for the high throughput analysis. Validation parameters for the Affymetrix miRNA122 assay were Intra-assay precision (11-28%CV), Inter-assay precision (7-40%CV), Inter-person precision (23-109%CV), Limit of Blank(948 copies/uL), Limits of Quantitation(1,111-111,111copies/uL), Dilutional Linearity, Freeze/Thaw Stability, Sample Storage Stability at 2-8C as well as -80C, Method Comparison and Biologic Correlation. For Biologic Correlation we evaluated miRNA122 as a biomarker of liver injury in a large number of clinical serum samples (N=563). This evaluation of samples collected from the University of Michigan health care system under an approved IRB included an assessment of healthy subjects across age and gender, subjects with a variety of liver impairments, and several cases of acetaminophen overdose. miRNA122 had a strong correlation with elevated ALT levels, and possessed a high predictivity for liver injury, as determined by ROC analysis. Our study demonstrates the utility of Affymetrix QuantiGene Technology for detection of miRNA122 in a clinical setting.

Notes

Author Index

A

A, M-A ... 1494
Aa, J ... 1953
Aaronson, J A ... 1611
Abafe, O ... 2922
Abam, E O ... 1249, 1527, 1547*, 1568
Abassi, Y ... 1890
Abassi, Y A ... 1740, 1741
Abbott, B D ... 2172
Abd Elmageed, Z Y ... 2333
Abdel Moneim, I ... 1585
Abdel Rasoul, G ... 2401, 2402, 2902
Abdelghany, T M ... 3399
Abdel-Mageed, A B ... 2333
Abdel-Rahman, S Z ... 2825, 3234
Abdelwahab, S ... 2907*
Abdou, K A ... 2914*
AbdulHameed, M D M ... 3140*
Abdullah, S ... 1938
Abelmann, A ... 1689, 1690*, 2140
Abiko, Y ... 1563*, 1582, 2295
Abo, T ... 2193*
Abo Norag, M A ... 2914
Abou Hadeed, A H ... 3257*
Abou-Donia, M B ... 2405, 2907, 2912*
Abplanalp, W ... 1836, 2134*
Abraham, R ... 1667
Abramson, M M ... 2281*
Abreu-Villaca, Y ... 1243*
Abreu-Villaça, Y ... 1164
Abritis, A ... 3000*
Abtout, S ... 2841, 2842*, 3105
Abudayyak, M ... 2761, 2785
Abukabda, A B ... 2817, 2820*
Acali, S ... 1106
Achanta, S ... 1301*
Acheson, S K ... 2907
Acikara, O B ... 1485
Ackerman, J ... 2655, 3157
Ackermann, G ... 1270
Acosta Amado, R ... 2208, 3546
Acquaah-Mensah, G K ... 1080*
Acuña-Hernández, D G ... 3223, 3228*, 3229
Ada, A O ... 2748
Adachi, R ... 2806
Adair, K ... 1315
Adamcakova-Dodd, A ... 2793*, 3425*
Adamou, A ... 1649*, 3096
Adams, R E ... 1300
Adams, T B ... 2279
Adamson, J ... 3037
Adamu, P ... 1462
Adebayo, S A ... 1433*
Adebisi, D O ... 1549*
Adebiyi, O E ... 1557*
Adebolu, O O ... 2801*
Adedapo, A A ... 2827*
Adedapo, A D ... 2827
Adedara, I A ... 1817*
Adeghate, E ... 1896
Adegoke, A M ... 1446
Adeleye, Y ... 1714*
Ademuyiwa, O ... 1248, 1249*, 1527, 1547, 1568
Adeogun, A I ... 1662
Adeogun, A O ... 3297*
Adeyemi, O O ... 1466, 1471
Adeyemo, O J ... 3019
Adeyemo, O K ... 2954
Adeyinka, O ... 2590
Adhikari, P ... 3165
Adkins, K ... 2481, 3467, 3480*
Adler, M ... 3118
Admon, S ... 3410
Adriaens, E ... 1614, 1616
Afolabi, O ... 3024
Afolabi, O K ... 1249, 1527, 1547, 1568
Afriyie-Gyawu, E ... 1685, 2282, 2284, 2590
Afzali, M F ... 1917*
Ag, M-C ... 1494
Aga, D ... 2356, 2902, 2969
Agarwal, C ... 2115, 2126
Agarwal, R ... 2115, 2116, 2121, 2126
Agbafor, U ... 1463
Aggarwal, M ... 2458
Aggerbeck, M ... 2430, 3427
Agier, L ... 3237
Agim, Z S ... 2372*
Agnihothram, S ... 2769*
Aguilar-Madrid, G ... 3130
Ahmad, S F ... 1343
Ahmed, R S ... 3227
Ahmed, S ... 1103
Ahn, H J ... 2940
Ahn, J ... 1424, 2149, 3429
Ahn, Y-A ... 2729*
Aho, J ... 1832*
Ahrens, L ... 2737
Aigbe, F R ... 1471*
Aioun, J ... 3237
Aisaki, K-i ... 2613
Ajibola, V ... 2932
Ajiboye, A O ... 3019*
Akagi, J-i ... 1467, 2598, 2806
Akahira, R ... 3148
Akande, M G ... 2913*
Akanni, G B ... 2922
Akbari, P ... 2873
Akhtar, M ... 2911*
Akinade, O A ... 2735
Akinbode, O A ... 1446
Akinhanmi, T F ... 1248, 1249
Akinmoladun, A C ... 3244
Akinosun, O M ... 1133*
Akinwumi, K A ... 1446, 2081
Akiyama, M ... 1582*, 2033
Akumka, D ... 1462
Akuse, S ... 2590*
Al Adhami, H ... 3237
Al Suliman, R J ... 1439, 3454*
Alam, G ... 2336*
Alam, M N ... 2127
Alarie, Y ... 2621*
Alava, P ... 1543
Alavi, A ... 3083
Albakheet, S ... 1343*
Albelda, S M ... 2047
Albert, I ... 2426, 2434
Albores, A ... 2956, 3219
Albornoz-Abud, N A ... 2953*
Alday, A ... 2456
Aldous, C ... 2900
Aldous, D ... 1093
Aleksunes, L M ... 1969, 2371, 2721, 3233, 3235, 3240, 3272, 3348*, 3350
Aleo, M ... 1073
Aleo, M D ... 2218
Alepee, N ... 1612*, 1613, 1614, 2206
Alépée, N ... 1616, 2534*
Al-Eryani, L ... 3340*
Alexander, D ... 3346
Alexander, P G ... 2178*
Alexander-Scott, M ... 1686
Alexandrie, A-K ... 2142
Alfaheeda, Z J ... 1937*
AlGhananeem, A ... 1315
Alghetaa, H F ... 1292*
Alharthy, K ... 3283*
Alhasson, F ... 1852, 1902, 1920, 1949, 3131*
Ali, M ... 1913
Ali, S ... 2347*
Aliberti, A ... 1835*
Aligo, J ... 1640*
Alimokhtari, S ... 1297
Alkelech, A M ... 3257
Alkhafaf, H ... 1272*
Allan, A M ... 1350
Allard, P ... 1812, 1839*, 2550, 3192
Allen, B ... 2240
Allen, D ... 1098, 2202, 2224, 2651, 2990
Allen, D G ... 1705*
Allen, J L ... 1166*, 1167, 1168, 1188
Allison, D B ... 2967
Allison, N ... 2714
Alm, K ... 3545
Almeida, M R ... 2593, 3016*
Almeldin, A A ... 3149*
Almond, K ... 2950*
Almy, F ... 1671, 1672
Alobaidi, R ... 1227
Alov, P ... 2656
Alpertunga, B ... 1321*, 2220
Alqassim, A Y ... 2058*
AlSadhan, L ... 3250
Alsop, D ... 3159
Altera, K ... 1626
Altincekic Gurkaynak, T ... 2785
Altmann, B ... 1606
Altomare, D ... 1797
Alugubelly, N ... 1170, 2414*
Alund, A ... 2318*
Aluru, N ... 2424
Alvarado, A ... 1423
Alvarez, R ... 1335
Alvarez-Ortega, N ... 1548
Alvear-Alayon, M R ... 2929*
Alyea, R ... 2468
Alzarie, Y A ... 3399
Alzualde, A ... 2456
Amaral, K ... 3145
Amaraneni, M ... 3447*, 3448
Amberg, A ... 1093, 2647*
Ambrosio, F ... 1250
Ameni, A Z ... 3463
Ameredes, B T ... 3039, 3155
Amin, R ... 1677, 1677
Amin, S ... 1328
Amin, S G ... 1733
Amiri, L ... 1896
Ammar, D A ... 2115, 2116, 2121
Ammendolia, D A ... 1100, 1118
Amos-Kroohs, R M ... 2368
Amy, O ... 1927
Anadon, A R ... 2278
Anadon, M J ... 2393
Anadón, A ... 2885*
Anand, S S ... 3448
Ananthanarayanan, A ... 1998, 3557*
Anantharam, P ... 1226, 2119, 2120*
Anantharam, V ... 2341, 2350, 2351, 2373, 2374, 2375, 2396
Anastas, P ... 2323, 2324
Andersen, J K ... 3356*
Andersen, M ... 1832, 2005, 2018
Andersen, M E ... 1084, 1119, 1251, 2014, 2038*, 2039, 2188, 2432, 2596, 2975, 3017, 3464
Anderson, A J ... 2757
Anderson, D ... 1513, 2125, 3196
Anderson, J M ... 3333b*
Anderson, K ... 1762b*
Anderson, K B ... 1524*
Anderson, S ... 2862
Anderson, S A ... 1642
Anderson, S E ... 1515, 2866*
Anderson, T M ... 1177
Anderson Daniels, T ... 1324
Andersson, N ... 2988*
Andes, M ... 3059, 3554
Andre, L ... 1148
Andreï, J ... 2805
Andres, D K ... 1323*
Andres, J F ... 1323
Andrew, K ... 2470
Andrew, M E ... 2538
Andrews, A ... 2447
Andrews, B ... 1658
Andrews, D ... 1066, 2135
Andrews, D L ... 1069, 1281
Andric, N ... 2306
Andriolo, J M ... 1507
Anestis, D ... 3109, 3139
Ang, K-H ... 1387
Anger, L ... 2647
Angermann, J ... 1266, 2259*, 2982
Angrish, M ... 1279
Angrish, M M ... 2213*
Ankley, G ... 1778b*, 2214, 2928
Anna, B-P ... 1148
Annalora, A J ... 2438*
Annand, B ... 3148
Annas, A ... 2966
Ansari, G ... 2870
Ansari, R A ... 1933, 2822*
Anson, B ... 2552b*
Anson, B D ... 1745
Antoine, D J ... 1994, 2727, 3129, 3178, 3472
Antoni, A ... 3167
Antonini, J M ... 1289
Antonio, R-U ... 1494*
Anttila, M ... 3008
Antunes, L M ... 1416, 2593, 3016
Antunes dos Santos, A ... 1322*
Apgar, K ... 1790
Api, A M ... 2644, 3442, 3519, 3520
Apostoli, A J ... 2083, 3094
Apreutese, A ... 3269
Apte, U ... 1373, 1952, 1971, 1977
Apte, U M ... 1964
Aptula, A ... 2644
Arıcı, M ... 2761
Arab, A ... 3222
Aragon, M ... 3485
Aragone, S ... 2901
Arantes, L ... 2357, 2358
Arasti, C ... 2470
Araujo, J A ... 1128*
Araújo, A M ... 1967
Are, P ... 1617
Arends, R ... 1238
Ares, I ... 2885
Arezzo, J ... 1208
Argos, M ... 2568*
Arguiri, E ... 2047
Arick, M ... 1806
Arita, A ... 2322
Arkin, M ... 1387
Armento, A ... 3069, 3516*
Armstrong, L ... 1956
Armstrong, R ... 3042
Arno, S G ... 2321
Arnold, L ... 1254
Arnold, L L ... 3184
Arnold, S ... 2226
Arnoldi, F G ... 2860
Aro, A O ... 1495*
Arosh, J A ... 3225
Arreola-Mendoza, L ... 3228
Arrigoni, C ... 2740, 2741
Arteel, G ... 3115
Arteel, G E ... 1984, 2211, 2496
Artik, N ... 1485
Artmann, G ... 2857
Arts, J H ... 2544
Arukwe, A ... 3297
Arumugam, V ... 3340
Arvidson, K ... 2163, 2467*, 2475, 2476, 2617
Arzuaga, X ... 2704*, 2705
Asahi, T ... 1931
Asakura, M ... 1957*
Asano, H ... 1092
Asare, Y ... 1447*
Ascah, A ... 2841*, 2842, 3105
Aschner, M 1322, 1580, 1581, 1817, 2357, 2358, 2361, 2362, 2364, 2365, 2379, 2512*, 3359*
Asghari, S ... 2940
Asgharian, B ... 2215
Ashby, C R ... 1916
Ashcroft-Hawley, K ... 2840
Ashikaga, T ... 2196, 2204
Ashmead, S ... 2457*
Ashton, R ... 1184
Aspegren, A ... 1997
Asquith, M ... 1899
Atchison, W ... 1576, 1577
Atchison, W D ... 1573, 1574, 1575, 1578
Atilano-Roque, A ... 3114*
Atosuo, J ... 3008

Atsuda, K ... 1957
Attalla, B ... 3440
Attanasio, R ... 2972
Attene-Ramos, M S ... 1811
Attia, S M ... 1343
Attignon, E ... 2430*
Attreed, S ... 1846
Aubrecht, J ... 2482, 3558
Aubrière, M-C ... 3237
Auerbach, S ... 1095, 1771*
Auerbach, S S ... 1451, 2864, 3528
Augsburger, A-S ... 1607
Augsburger, M ... 3403
Aujean, E ... 3237
Auta, J ... 2932
Authier, S ... 1208*, 2841, 2842, 3105, 3243
Autrey, J J ... 1601
Avalos, J ... 2193
Avlasevich, S ... 1061, 1410
Awad, A S ... 2874
Awad, N E-H Y ... 1210*
Awoufack, M D ... 1495
Awoyemi, O M ... 1430*
Ayanda, I ... 2932*
Ayehunie, S ... 3069*, 3070
Aylor, D L ... 2546*
Azevedo, M ... 2769
Azizi, E ... 2355
Azzopardi, D ... 3037

B

Baas, H M ... 2589*, 3009*
Babarinsa, O S ... 2827
Babayemi, D ... 1248
Babayemi, D O ... 1249, 1568
Babel, A ... 3024
Babica, P ... 2308
Babin, M ... 2103
Babin, M C ... 2093*, 2102, 2404
Babin, P J ... 1806
Babu, S ... 1623, 2314*
Baccarelli, A ... 1723*
Baccarelli, A A ... 1432
Bach, N ... 1450
Bachelor, M ... 3066, 3516
Bachus, S ... 3085
Backes, W L ... 1303
Bacolod, M ... 3437
Badary, O A ... 3399
Badawy, N ... 2387
Badding, M ... 1314*, 2780
Badger, T M ... 1874
Bae-Jump, V L ... 3231
Baer, C E ... 1541
Baer, M R ... 2048
Baertsch, N A ... 1177
Baeyens, W ... 1142
Baeza Terrazas, F ... 1261, 1262
Bagam, P ... 1932*
Bagchi, D ... 2587*
Bagchi, I C ... 1914
Bagchi, M K ... 1914
Bagley, D ... 2676
Bai, S ... 3198
Bai, T ... 2833*
Bailey, G P ... 2563*
Bailey, J ... 1848
Bailey, K ... 1259*, 1262
Bailey, M A ... 3277*, 3472
Bailey, R A ... 2368*
Bainy, A C ... 2949
Baird, C ... 2145
Baird, R ... 2442
Bais, M ... 1732
Bajaj, N ... 2180
Baker, A ... 1883
Baker, A A ... 1969
Baker, E S ... 1136
Baker, G L ... 1308
Baker, N C ... 2547, 3300*
Baker, T ... 2197, 2516*
Baker, T K ... 1108, 1739
Baker, T R ... 1185
Bakhiyi, B ... 2693
Bakke, J P ... 1626
Balachandran, R ... 1230
Balachandran, Y ... 2805
Balagopal, G ... 1454*
Balamurali, G V ... 2212
Balbuena, P ... 1413, 1867, 1871*
Baldrick, P ... 1629*, 2562b*
Baldwin, W S ... 3156
Balestra, A ... 1146
Ball, J G ... 3126
Ball, N ... 2527*, 2711
Ball, N S ... 1426
Ball, R W ... 3298
Ballet, V ... 2647
Ballinas-Casarrubias, L ... 1261, 1262
Balog, R ... 2107
Balogun, E A ... 1527, 1547, 1568, 1662*
Baltzley, S ... 1315
Bam, M ... 1943*
Bammler, T K ... 3270
Ban, I ... 1405, 1428*, 1919
Bañares, M ... 2775
Bandara, S ... 2105*
Bandiera, S M ... 1873, 3411*, 3412
Banducci, A ... 2273
Banerjee, A ... 2808, 3037
Banerjee, B D ... 2910, 3227
Bannish, G ... 1631
Bannuscher, A ... 1518
Banton, M I ... 2036, 2038
Banton, S ... 2319, 2730
Banton, S A ... 1529
Banu, S K ... 3225*
Baptista, P ... 1963
Barbeau, D ... 1146*
Barbier, O ... 3134
Barbier, O C ... 3130
Barchowsky, A ... 1250
Bare, J L ... 1689
Barenys, M ... 1082
Barfuss, D W ... 3120
Barger, M ... 1314, 1496
Barham, R ... 1685
Barhoumi Mouneimne, R ... 3392
Barile, F A ... 1835
Barillo, D ... 2110, 2111*, 2123, 2124
Barker, J ... 2924
Barletta, M ... 2330
Barlow, S ... 2475
Barman Michelsen, V ... 2445
Barnes, A ... 1561
Barnes, E ... 1844
Barnett, A ... 1201
Barnett, H ... 1439
Barney, D L ... 1490
Barouki, R ... 2743, 3427
Barr, D B ... 1529
Barrett, E S ... 1166
Barrett, J ... 2069
Barrett, K G ... 3457*
Barrie, M ... 2520*
Barrios Payán, J ... 1978
Barron, K ... 1261
Barroso, J ... 1616
Barski, A ... 2322
Bartels, M ... 2226
Bartels, M J ... 2458
Bartels, M M ... 2674
Barthlow, H ... 3069
Bartlett, C ... 2137
Bartlett, M G ... 2897
Bartoe, J ... 1605
Barton, H A ... 2218*
Barton, S ... 2924
Barton-Maclaren, T ... 1769*
Barve, S ... 1976
Basaure, P ... 2915, 2916
Bashandy, M ... 1481
Bass, V ... 1067, 2823*
Bassaganya-Riera, J ... 1659
Bassan, A ... 2476
Bassett, S ... 1644
Bast, A ... 3015
Bastos, M D L ... 1967*
Bastos, M L ... 1963
Basu, N ... 3027
Bataille, A ... 2434
Batdorf, S ... 2974
Bateman, D N ... 2727, 3472
Bathula, C S ... 3127
Batke, M ... 2650, 3081
Batra, S ... 1908, 1932, 2290
Battelli, L ... 2538, 3485, 3488
Battelli, L A ... 1199
Battistoni, M ... 3279, 3280
Bauer, A K ... 1444, 1907
Baumer, K ... 1106, 3035
Baumgart, B ... 3477
Baumgart, B R ... 1332*
Baustian, J ... 2459
Bauter, M R ... 3011*
Bautista, A C ... 1197
Bautista, M ... 2283
Baxter, A ... 3037*
Baynes, R ... 3435
Bazany, G ... 1577
Beach, T ... 1290*
Beach, T A ... 1903
Beamer, C A ... 1361, 1367
Beasley, A ... 2226
Beaton-Green, L A ... 3533
Beattie, P J ... 2615*
Beaudin, S ... 1230
Beaudoin, S ... 3101*
Beausoleil, J ... 3493
Beauvais, S ... 2900
Becerra-Romero, W E ... 2325
Becher, R ... 2443, 2444*
Beck, A ... 2442
Beck, B D ... 1692
Beck, N ... 2166
Beck, T ... 3090, 3095, 3099, 3277
Becker, N ... 1745, 1829
Becker, R ... 1768*, 2677*
Becker, R A ... 2468
Beckerman, B S ... 2979*
Beckett, E M ... 1300, 1689*
Beckler, M ... 1829
Bednar, A J ... 3454
Beebe, D J ... 3266, 3388
Beetsch, N ... 2155*
Beevers, C ... 1409*
Beger, R D ... 1110, 2642*
Beggs, K M ... 1952*
Begley, C G ... 1035b*
Behar, R Z ... 2480*
Behl, M ... 1013*, 1353, 1609, 2456, 2585*
Behm, D J ... 1301
Behr, C ... 1115*
Behrns, K ... 3169
Behrsing, H P ... 2175*
Beier, E ... 2348*
Beier, J I ... 1984
Beilstein, P ... 3004*, 3005
Belair, D ... 3080
Belair, D G ... 2172*
Beland, F A ... 1059, 1642, 2037, 2084, 3193, 3208, 3455
Belanger, P ... 1238*
Beliveau, M ... 2108*
Bell, D ... 2307
Bell, D A ... 1837, 3200
Bell, D R ... 2988
Bell, J A ... 2875
Bell, M P ... 2893
Bell, P D ... 1920
Bell, S M ... 1101
Bellary, S ... 1735
Belleggia, G ... 3405
Bellés, M ... 2315
Bellissima, B ... 2828*
Bello Monroy, O ... 1978
Belton, K ... 2417*
Beltran, M ... 2455
Bemis, J ... 1060, 1410
Bemis, J C ... 2639*
Benbrahim-Tallaa, L ... 2062*
Benderitter, M ... 2688
Bendt, F ... 1161
Benjamin, B L ... 3024*
Bennett, B ... 1837
Bennett, J A ... 1837
Beno, O O ... 1549
Benoît-Biancomano, M ... 3269
Benson, K ... 2970*
Benson, S ... 2974*, 2976
Bento, P ... 2650
Benton, B ... 2107
Benton, B J ... 1323
Bentz, S ... 3259
Beranek, V ... 1438
Bereman, M ... 3490
Béres, E ... 1457
Berg, C ... 2374
Berg, E L ... 3386*
Berg, F ... 2117
Berg, K ... 2591
Berger, F ... 1852
Berger, F I ... 2043
Bergeron, A B ... 1990*
Berhanu, A ... 2108
Berlien, H-P ... 3525
Bernacki, D ... 2639
Bernaki, D ... 1060
Bernal, A ... 2695
Bernardi, C I ... 2740, 2741*
Bernhard, M C ... 2967*
Bernhard, M J ... 2925
Berthet, A ... 3403*, 3515
Bertinetti-Lapatki, C ... 1828
Bertotto, L B ... 2941*
Bertrand, A ... 2937
Besisi, J ... 3495
Bessa, M J ... 2775
Besselink, H ... 2431
Betancourt, D ... 1791
Betancourt-Aldazaba, M L ... 2325
Bevan, C J ... 1179
Beverly, L ... 3115
Beverly, L J ... 2496
Beverly, S ... 3025
Beyer, D ... 1138
Beyer, L A ... 2270*
Beyerle, A ... 2808
Bhalli, J ... 1402
Bhandari, S ... 1936*
Bharadwaj, M ... 3108*
Bhardwaj, G ... 1619
Bhaskaran, V ... 1668, 2726, 3091*
Bhat, V S ... 1372*, 2138, 2152, 2155
Bhatnagar, A ... 1836, 2134, 2250
Bhatt, A J ... 1232, 2345
Bhatt, S ... 1238
Bhattacharjee, P ... 2052
Bhattacharya, S ... 1119*, 2210
Bhattacharyya, S ... 1110, 2489
Bhhatarai, B ... 2191*, 2226
Bhushan, B ... 1964, 1971*, 1977
Bi, H ... 1597
Bianchi, M D L ... 1416
Bianchi, M L P ... 2593, 3016
Bibi, H ... 2821
Bicakcioglu, P ... 2748
Bichell, T J ... 2379
Bichell, T J V ... 2382
Bichlmaier, I ... 2988
Biedrzycki, J ... 1299
Bienfait, B ... 2467, 2657, 2709
Biesiada, J ... 1537
Bigge, K ... 1627
Bijegaonkar, U ... 1617
Bijli, K M ... 2360
Bijol, V ... 3123, 3138
Bilgesu, S ... 3485
Bilgo, D ... 3532
Billack, B ... 2891
Bind, M-A ... 1071
Binks, M ... 2482
Bircsak, K ... 3272
Bircsak, K M ... 3233, 3235*
Birkebak, J ... 1925*
Birnbaum, L ... 1143, 2733
Birnbaum, L S ... 1051b*, 2045, 2995, 3406, 3419, 3528
Birukova, A ... 1064
Biryol, D ... 1682
Bischof, I ... 2925
Bisesi, J H ... 2811
Bishop, L ... 1701, 2782, 3485, 3496
Bishop, L M ... 3488*
Bishop, M E ... 1396
Bissel, P ... 1659
Bisson, W H ... 2419, 3285, 3538*
Biswajit, M ... 2108
Bitragunta, S P ... 1501*
Bitsch, A ... 3081*, 3548
Bittner, M ... 2711
Blaauboer, B ... 2530*
Black, K ... 1297

Black, M ... 2038, 2164, 2422
Black, M B ... 2039*
Black, S ... 2602, 3453
Blackburn, M ... 1874
Blackman, B ... 2006
Blackwell, B R ... 2928*
Blain, R ... 2168
Blair, I ... 2317
Blair, I A ... 1312
Blais, J M ... 1218
Blake, D J ... 1337*
Blakeman, L ... 1615
Blakley, B ... 2991
Blakley, B R ... 2831
Blanc, E ... 2430
Blanchet, D ... 2625
Blanco, J ... 2795
Blau, T S ... 2073, 2894*
Bleumink, R ... 1334
Blömeke, B ... 2863, 3517
Bloom, M S ... 2571*
Blossom, S ... 3198
Blossom, S J ... 1196*
Blount, B C ... 3241
Blum, J ... 1167, 1846, 3248
Blum, J L ... 1847
Blystone, C R ... 3273, 3444, 3453
Bobczynski, E M ... 1875
Bober, M ... 2000
Bodar, C ... 2718
Bodnar, W ... 2086, 2977, 3434
Boekelheide, K ... 1084, 2174, 3302*
Boere, J A ... 3237
Boes, K ... 1659
Boge, L ... 1941*
Bogen, K T ... 1254, 2661, 2905*
Boggess, K ... 2977, 3199
Bohic, S ... 2772
Bohlen, H ... 2857
Boisclair, J ... 2605
Boitel, E ... 2199
Boland, S ... 2810
Bolden, A L ... 1859
Boles, C ... 1843
Bølling, A K ... 1450
Bol-Schoenmakers, M ... 1334, 2873*
Bolser, D ... 3495
Bolt, A M ... 1554, 1555*
Bombick, B ... 1325
Bommegowda, S K ... 1893*
Bondesson, M ... 1794, 1807, 3286
Bone, A J ... 1848, 3038
Bonini, M ... 1025*
Bonk, J ... 2659
Bonn, M ... 2877, 2878
Bonnel, D ... 1293, 1983
Bonner, J ... 2782, 3490
Bonner, J C ... 3494, 3497, 3498, 3500, 3502, 3503
Bonner, J S ... 1701, 3507
Bonner, M ... 2902
Bonner, M R ... 2401, 2402
Bonzo, J ... 1974, 1987
Bonzo, J A ... 2493
Boobis, A ... 1036*, 2475, 2685
Booker, B ... 1946
Boonen, K ... 3460
Boor, P J ... 2177
Boosalis, C A ... 1229*
Boots, A W ... 3046
Borders, R B ... 1625, 2856
Bordner, A ... 2168
Borges, M ... 2108
Borges, V R ... 1875
Borghoff, S ... 3407, 3445*
Borghoff, S J ... 3089
Borgne-Sanchez, A ... 2015, 3549*
Börjel, M ... 2171
Bornhorst, J ... 2365
Borrás-Pacheco, T M ... 1383*
Borsay Horowitz, D ... 2933
Borude, P ... 1977*
Borunda, T ... 1324
Bory, C ... 2835, 2837
Boss, G ... 2120
Boswell, W S ... 3156
Bot, C T ... 1829*
Botehlo, D ... 2328
Botham, J ... 2680
Botta, D ... 1499
Bottai, M ... 1944
Bottomley, A ... 1634, 2839
Bou Aram, B ... 3152
Bouchard, G ... 1237, 3082, 3084*, 3088, 3513
Bouchard, M ... 1144*
Bouchard, P ... 2605
Boudreau, M ... 1514
Boudreau, M D ... 2799*
Boue, S ... 3401, 3402
Bouhifd, M ... 1084, 3408
Bouitbir, J ... 1972
Bouknight, S A ... 1857
Boulange, C L ... 1107
Boule, L ... 1368
Boule, L A ... 1365*
Boulos, N ... 2695
Bounous, D ... 3091, 3256
Bourbonnais, R ... 2693
Bourdi, M ... 3092
Bourgeois, M ... 1372, 1384, 2854, 3174
Bovee, T F ... 1480
Bowers, E ... 3220*
Bowers, E C ... 3215
Bowler, R ... 1596
Bowman, A ... 2379, 3357*
Bowman, A B ... 2339, 2358, 2364, 2365, 2382, 2553*
Bowman, C J ... 1801, 2551*, 2565*
Boyd, J ... 1441, 1922*
Boyd, W ... 2179
Boyd, W A ... 1811, 2294
Boyer, I ... 2476, 2709
Boyer, J ... 2739
Boyer, J A ... 1733
Boyer, J L ... 1966
Boyes, W K ... 1033*, 2765, 3332*
Boylan, M ... 2465*
Boysen, G ... 1122*, 3155
Bozhilov, K ... 3032
Braam, S R ... 1744, 1830
Braber, S ... 2873
Bradfield, C ... 2076
Bradham, K ... 1543, 2235
Bradley, E ... 2379
Bradley, E M ... 2382
Bradley, J ... 2850
Bradley, J A ... 1155*, 2495
Bradley, L J ... 3002*
Bradley, M A ... 3027*
Bradner, J M ... 3247, 3253
Brandwein, D ... 2189
Brannen, K ... 3256
Bratasz, A ... 2856
Bratz, M ... 3553
Braubach, P ... 2710
Braun, A ... 2710
Braun, E ... 3486
Braydich-Stolle, L K ... 3504
Brayman, T ... 2011*
Breckenridge, C B ... 2917
Bredfeldt, T ... 2158*
Breheny, D ... 2053*
Brehm, E ... 1855
Breier, A ... 2008
Breitenstein, M J ... 1686
Brennan, M B ... 1626
Brennan, R J ... 1093*
Brenner, S ... 2539
Breton, L ... 1228, 2007, 2477
Breyfogle, B ... 3066
Brickel, J A ... 2154*
Bridge, M F ... 1811
Bridges, C ... 3120*
Bridges, J ... 2703
Bridges, L R ... 2007, 2477
Briens, J M ... 3010*
Briffaux, J-P ... 2835, 2837
Briggs, K ... 2641
Briggs, K A ... 2630*
Brigo, A ... 2641
Briley, A ... 3117*
Brinchmann, B C ... 1450
Brinkerhoff, C J ... 2700
Briotet, D ... 3547
Brito, L ... 3296
Brittain, M ... 3106
Brittain, M K ... 2102*
Brix, A ... 2131
Brix, A E ... 3177
Broadfoot, B ... 3198
Broberg, K ... 2509*
Brocato, J ... 2064, 2072
Brock, B ... 3479
Brock, G ... 3170
Brock, G N ... 2211
Brock, M ... 1743*
Brock, W J ... 3175
Brocker, C ... 1987*
Brocksmith, D ... 1237
Brodbeck, C ... 3548
Broderick Pritty, M ... 3352
Brodie, T ... 1676
Brodsky, B ... 2098
Broening, H W ... 1805
Brohem, C ... 2474
Bronson, R ... 3028
Brooks, B ... 2323, 2324
Brooks, D ... 3396
Brooks, J ... 3435
Brooks, P T ... 2875*
Brooks, S ... 1533*
Brorby, G ... 1542*
Brosnan, K ... 1640
Brower, J ... 1114, 2261
Brown, A ... 1685*
Brown, D ... 2096
Brown, D G ... 1081*
Brown, D L ... 3275, 3276
Brown, J ... 1150*, 1181, 2262
Brown, J M ... 1502, 1520, 2767, 3503
Brown, K ... 1382
Brown, L ... 1162*
Brown, M M ... 3048
Brown, P ... 2455
Brown, R ... 2678*, 2719
Brown, S C ... 2543
Brown, S E ... 3292*
Browne, P ... 1726b*, 2651
Browning, C L ... 2926*
Bruce, S W ... 1398, 2053
Bruckers, L ... 1142
Bruckner, J V ... 2897, 2908, 3447, 3448
Brüggemann, A ... 1829
Brun, E ... 2791
Brunnemann, K D ... 1820, 1821
Brunner, J ... 2541*
Brunner, T ... 2605
Bruno, V ... 1408*
Bruun, D ... 1229, 2409, 2410
Bruun, D A ... 2395*
Bruun, J-A ... 2445
Bryan, M R ... 2379*
Bryant, M S ... 2799
Bryant, S ... 1671
Bryce, S ... 1060*, 2639
Bucher, J R ... 3528*
Buchholz, B A ... 1288
Buchi, D F ... 2860
Bucio, L ... 1973, 1978
Buck, M ... 2051
Buckalew, A R ... 1887*, 1888, 1889
Buckley, B ... 1135, 2242, 2371, 2400
Buckman, K ... 2937
Budinsky, R ... 1736, 2422
Budinsky, R A ... 1138
Bugarski, A D ... 2803
Bugel, S M ... 1121, 1805*
Bujalowski, P ... 2177
Bujold, K ... 2841, 2842, 3105
Bull, T M ... 1875
Bullins, K W ... 2720
Bunch, R T ... 1332
Bunker, K ... 2773, 3488
Buno, K ... 2180
Burbacher, T ... 3278, 3446
Burban, A ... 1970
Burbank, M ... 1970
Burchett, J ... 2779*
Burchiel, S W ... 1350, 1352
Burden, N ... 2927*
Burgess, J ... 3453
Burghardt, R C ... 3225
Burgoon, L ... 2643*
Burim, R V ... 1416
Burin, G ... 2909*
Burleson, F ... 1925
Burleson, F G ... 2864
Burleson, G R ... 2864, 3376*
Burn, B ... 2850
Burn, B R ... 2331*
Burnett, D ... 2459*
Burnett, S ... 3193, 3208*
Burns, A M ... 2672*
Burns, F ... 1124
Burns Naas, L A ... 2957*
Burns-Naas, L A 1925, 2826, 2829, 3375*
Buron, N ... 2015, 3549
Burra, N L K ... 1363*
Burrell, K L ... 2599
Burris, D ... 1414*
Burris, H H ... 1432
Bursley, J ... 1346*
Burt, D ... 3558*
Burton, E ... 2377
Burton, J ... 3531
Burwell, S ... 1848
Bus, J S ... 1179*, 2036, 2038
Busbee, P B ... 1900*
Busch, J ... 2478
Buscher, B ... 1138
Buse, J ... 1262
Busque, M-A ... 2693
Busquet, F ... 2632
Bustamante, A ... 2904
Butler, P ... 1238
Butt, M ... 1238, 1641
Büttner, A ... 3040
Buxton, W ... 1862
Buzzo, M L ... 1553
Byloos, M ... 2723

C

Caballero-Gallardo, K ... 1548, 2946*
Cable, P H ... 2977
Cabré, M ... 2915, 2916
Cabrero, P ... 1271
Cachot, J ... 1806
Cadavona, J J P ... 3510
Cade, S ... 1697*, 3404
Cai, D ... 1329
Cai, J ... 2304, 2906, 3430*
Cai, L ... 1879, 2600, 2601, 2833, 3125
Cai, Q ... 3202
Cai, Y ... 1228, 2477
Cain, G ... 1666
Caito, S W ... 1569*
Cajero, M ... 1132
Calderón-Garcidueñas, A L ... 2325
Caldwell, D ... 3028
Calhoun, M ... 1269
Callander, R ... 2685
Callegaro, G ... 2291
Callejas, Y ... 1668
Callol, C ... 2942
Calton, M A ... 3418
Calvano, J ... 3477, 3478*, 3479
Calvo, N S ... 1845
Camacho, J ... 1839
Camacho, J A ... 3192*
Camacho, L ... 3470, 3471*
Camacho, O ... 3040
Camacho, O M ... 2053, 3041
Cambier, S ... 1511*, 2805
Cameán, A M ... 3020, 3022, 3023
Camenisch, T ... 1015*
Camenisch, T D ... 3345
Cameron, E ... 2354
Camous, S ... 3237
Campagnolo, L ... 1773*
Campbell, A ... 1217
Campbell, J ... 2240*
Campbell, J A ... 1407
Campbell, M ... 2307, 3200
Campbell, M E ... 2024
Campbell, M R ... 1837
Campen, M ... 1114*, 1309, 2261, 3485
Campen, M J ... 1304
Camus, S ... 3549
Canales, K K ... 2352
Cananzi, S G ... 2384

The numerals following the author names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the presenter.

Cañas-Carrell, J E ... 1385
Canatsey, R D ... 2082*
Canavez, A D ... 2474*
Canerdy, T D ... 2473, 2882
Canet, M J ... 3179
Canipa, S J ... 1399*, 2628, 2629
Cannon, J ... 1019*, 1022*, 2366, 2376
Cannon, J R ... 2372
Cano-Sancho, G ... 1850
Cantrill, C ... 1635*
Cao, S ... 2054
Cao, Z ... 3013
Caporali, A ... 3472
Cappon, G D ... 1801, 2551
Caputo, F ... 2807
Carano, R ... 1638
Cararo, G A ... 2474
Carbo, A ... 1659
Card, J W ... 2150*, 2151, 2667
Cardenas Gonzalez, M ... 3134*
Cárdenas-Gonzalez, M D C ... 3130
Cárdenas-González, M ... 3123
Carey, A ... 2826, 2829
Carey, C ... 1828
Carfagna, M A ... 1648*
Carlin, D J ... 2567*
Carll, A P ... 2855*
Carlsen, K-H ... 3258
Carlson-Lynch, H ... 1436, 2708
Carman, J ... 1676
Carmany, D ... 2117
Carmichael, P ... 2174, 2212, 3051, 3305*
Carmichael, P L ... 2596, 3071
Carmo, H ... 1443*
Carney, E W ... 2191
Carns, N E ... 2546
Caro, A A ... 3155
Caron, A ... 1624
Caron-Beaudoin, E ... 3393*
Carpio, V ... 1351
Carr, G ... 1805
Carr, R L ... 1170, 2414
Carr, T ... 3030
Carratt, S A ... 1288*
Carreira, V S ... 1296
Carrio, P ... 2684*
Carroll, E E ... 1540*
Carroll-Turpin, M ... 2844
Carson, J ... 1852
Carswell, G ... 1056
Carter, D E ... 2428*
Cartlidge, G ... 2988
Cartron, G ... 2483
Cartularo, L A ... 1562
Cartus, A T ... 2591*
Carty, D R ... 3282*
Carvajal, I ... 3440
Carvalho, A ... 1963
Carvalho, F ... 1963*, 1967
Carvalho, M ... 1967
Carvalho, M D F H ... 1553
Carver, Z A ... 1129, 1130, 1131*
Cascio, W E ... 2263
Case, K ... 2882
Cases, M ... 2641*
Casey, B J ... 2449, 2764
Casey, W ... 1098, 2202, 2224, 2651, 2990
Casillas, R ... 1373
Casillas, R P ... 2097, 2099, 2116, 2121
Casim, E ... 1235
Cassee, F R ... 1772b*, 3237
Casson, L ... 2496
Castellano, V ... 2885
Castle, K ... 2596
Castranova, V ... 2538, 2800, 2830, 3485, 3496
Casuccio, G ... 3488
Catlin, N ... 3261*
Caudle, W M ... 3247, 3253
Cauley, M ... 1164
Causey, R D ... 1601*
Cave, M ... 1962, 2733, 3170
Caverly Rae, J ... 1124
Cawley, M ... 2167*
Cayley, A ... 2619
Cayzac, C ... 1636
Cebrian, M E ... 1260*
Ceccatelli, S ... 2807
Cecil, A ... 1116
Ceger, P ... 2224, 2651
Celestin, N ... 2053
Celik, S ... 2748
Cemiloglu Ulker, O ... 3408
Cena, L ... 2773, 3488
Cercillieux, A ... 1611*
Cerniglia, C ... 1514, 3026
Cerreta, J M ... 2798, 2801
Cervelli, J ... 1910, 2118
Cesta, M ... 2131
Cesta, M F ... 1308
Chadalapaka, G ... 2056
Chadha, P ... 1465
Chadwick, A E ... 3129
Chadwick, K D ... 1332
Chadwick, L H ... 1724*
Chalansonnet, M ... 2791
Chalencon, E ... 2835, 2837
Chambers, C ... 2938
Chambers, D M ... 3241
Chambers, H ... 2090
Chambers, H W ... 2091, 2092, 2109
Chambers, J ... 2090
Chambers, J E ... 1877, 2091, 2092, 2109, 2725
Chambers, M ... 3111
Chambers, W A ... 1034*
Champeil, E ... 1316
Champlin, D ... 2937
Chan, C ... 2277
Chan, C Y ... 2671*
Chan, E ... 2242*
Chan, H M ... 1218
Chan, K ... 3514
Chan, K-M ... 1860, 1861*, 3459
Chan, M ... 1402, 2415*
Chan, S ... 1027*
Chandel, N ... 3065
Chandler, J ... 1125, 2319
Chandler, J D ... 1529, 2326*, 2360, 3421
Chandler, K ... 1822
Chandler, K J ... 2995*
Chandramouli, B ... 1780*
Chandran, P ... 2760*
Chandrasekhar, V ... 2855
Chandrashekaran, V ... 1852*, 1902, 1949
Chang, C-W ... 3470, 3471
Chang, D ... 1009*, 1611, 2221, 2238
Chang, E T ... 2979
Chang, H-Y ... 2112
Chang, J ... 2060, 2450
Chang, J H ... 1643
Chang, J-F ... 1345*
Chang, S-Y ... 1105*
Chang, W L W ... 1366
Chang, X ... 2224*, 2963
Chang, Y ... 1600
Chang, Y R ... 1169*
Chang, Y-C ... 2112*
Chang, Y-J ... 1412*, 2071
Chantry, M ... 2249
Chao, M-W ... 1182, 1345, 1412, 2071, 2383
Chapleau, R R ... 2217
Chapman, A M ... 2978*
Chapman, E ... 2602
Chapman, K ... 1634
Chapman, S ... 2129
Chappell, G ... 3190*, 3433
Chappell, V ... 3271
Chaput, D ... 1223
Charkoftaki, G ... 2752
Charla, S ... 1791, 1822
Charlap, J H ... 1179
Charlebois, M ... 1418
Charles, P ... 1199
Charli, A ... 2341*
Charlier, M ... 3237
Charrière, N ... 3515
Chatfield, R ... 2470
Chatham, L ... 2068, 2436*
Chatterjee, N ... 1512*, 3217
Chatterjee, S ... 1852, 1902, 1920, 1949
Chattopadhyay, A ... 2594
Chatziioannou, C ... 2985
Chau, B ... 2602
Chau, J ... 1387
Chaudhuri, I S ... 3496
Chavan, H D ... 3341*
Chavatte-Palmer, P ... 3237
Chavez-Bautista, M ... 2269
Chawade, A ... 2205
Chebekoue, S ... 1096*
Checkoway, H ... 2352
Cheeseman, M ... 2163
Chege, A W ... 2389*
Cheifetz, I M ... 1301
Cheikhi, A ... 1250*
Chelsky, D ... 3041
Chen, A ... 1797
Chen, B T ... 1497*, 2538
Chen, C ... 1656, 2937
Chen, D ... 1656, 2064, 2072, 3201, 3338*
Chen, F ... 2435
Chen, G ... 1160*
Chen, H ... 1191, 1193, 1197*, 2787, 2811*, 3495
Chen, J ... 1831, 1831, 2304*, 2366
Chen, J D ... 2383
Chen, L ... 1275, 1478, 1993*, 2024, 2029, 2302, 2843, 2906*
Chen, L C ... 1687
Chen, L-C ... 1846, 1847, 2246, 2247, 2254
Chen, M ... 1091, 2479*, 3098, 3168*, 3183, 3187
Chen, P ... 2365*
Chen, Q ... 2064
Chen, Q Y ... 2072, 2075*
Chen, R ... 1084, 1631, 2843
Chen, S ... 2012, 2592, 2747, 3450, 3455
Chen, S-J ... 2726
Chen, T ... 1059, 2037, 2435*, 2758*, 3205
Chen, T-h B ... 1921, 3485
Chen, W ... 1694, 1810, 2029*
Chen, W-Y ... 1976*
Chen, X ... 1072, 1892, 2051, 2888*, 2944, 2969*, 3073*, 3514
Chen, X-S ... 2061
Chen, Y ... 1093, 1189*, 1410, 1589, 1653, 1671, 1672*, 1812*, 2309, 2462*
Chen, Y-F ... 2844*
Chen, Z ... 2177*
Cheneval, E ... 2693
Cheng, H ... 1601, 1619
Cheng, S L ... 2754*
Cheng, S-Y ... 1316, 1377*, 2890
Cheng, W ... 2210, 2214*
Cheng, W-Y ... 1102
Cheng, X ... 1993, 2302, 3180
Cheng, Y ... 2026*, 2066, 2600*
Chengen, Z ... 2479
Cheong, H-K ... 3217
Chepelev, N ... 2088*
Chernick, M ... 1483, 2888, 2944
Cherr, G ... 1174
Cherrington, N J ... 1985, 3179, 3345
Cherry, S ... 2409
Chesne, C ... 2173*
Chesné, C ... 3549
Chester, A ... 2826, 2829
Chevalier, G ... 3259*
Chilton, M L ... 2628*
Chipman, J K ... 3205
Chirino, Y I ... 2790
Chitrakar, R ... 2821
Chitrala, K N ... 1076, 1085*
Chitranshi, P ... 3455
Chiu, J-Y ... 1182
Chiu, N H ... 2763*
Chiu, W ... 3433, 3434
Chiu, W A ... 2700, 3363*
Chlebowski, A ... 3285*
Cho, M-H ... 1464, 2880
Cho, T J ... 1505
Cho, Y H ... 3501
Cho, Y-M ... 1467, 2598, 2806*
Chockalingam, A ... 3473
Choi, B ... 1890
Choi, C Y ... 1470
Choi, H ... 1910
Choi, H J ... 1942
Choi, J ... 1512, 2310, 3217
Choi, J H ... 1460, 1469*, 1470
Choi, J K ... 1468, 1517
Choi, J S ... 2948
Choi, K ... 3078*, 3079
Choi, M M ... 2763
Choi, Y-J ... 1233, 1474
Choksi, N ... 2202, 2224
Cholanians, A B ... 1244, 1599*, 2380
Cholger, D ... 2363*, 2366
Chong, W ... 1595
Chopra, R ... 1018*
Chorley, B ... 1056, 1102
Chorley, B N ... 1407, 2213, 2616
Chou, C ... 1666
Chou, C-E ... 2127
Chou, S ... 3098
Choudhury, D R ... 1084
Chowdhury, A ... 1254, 3184*
Christian, B ... 1608
Christian, W V ... 2281
Christofidou-Solomidou, M ... 2047
Christudoss, P ... 3341
Chu, C ... 3152, 3158
Chua, A S ... 3123
Chuah, J K ... 3075
Chuang, G ... 2850
Chuang, Y-C ... 1182*, 2383
Chukwu, L O ... 1277
Chukwu, O L ... 3424
Chumbow, A ... 2059*
Chun, D S ... 1735
Chung, A ... 1904
Chung, H ... 1873*
Chung, K H ... 1287, 1306
Chung, Y C ... 1460, 1469, 1470*, 1488
Church, R J ... 3476*
Churchill, G ... 2753
Churchwell, M I ... 1139*
Chushak, Y ... 1094
Ciccimaro, D ... 1312
Cichocki, J A ... 3433*
Cipriano, R ... 3067
Cirrilo, P ... 2730
Cizdziel, P E ... 3148*
Clair, H ... 2733
Clair, H B ... 1962, 3170*
Clapp, P W ... 1338*
Clark, A ... 1839, 3192
Clark, B W ... 2937*
Clark, J M, ... 2889
Clark, J M ... 2588, 2881
Clark, K ... 2178
Clark, O C ... 1323
Clark, R D ... 1099
Clark, R J ... 3153
Clark, R S ... 1213
Clarke, E ... 1833*
Clarke, J D ... 1985, 3179
Clarke, J I ... 3472
Clarkson-Townsend, D ... 3247, 3253*
Claude, N ... 1970
Claville, M O ... 1623, 2314
Clements, J ... 2923*
Cleves, M A ... 1874
Clewell, A ... 1457*, 1458, 1459
Clewell, H ... 2188, 2240, 2701, 2975
Clewell, H J ... 1413, 2014, 3410, 3464
Clewell, R ... 1711*, 1730*, 2005, 2018
Clewell, R A ... 1413, 1867, 1871, 2432, 2596, 3071
Cline-Fedewa, H ... 1951, 1954
Clippinger, A J ... 1710*
Close, D ... 3556
Cloutier, M M ... 1295
Clouzeau, J ... 2625
Coarfa, C ... 3143, 3465
Coban, T ... 1485
Coburn, J ... 2450, 2453, 3162
Coburn, J L ... 1206*
Cochran, R E ... 1450
Coecke, S ... 1715*
Coffin, S L ... 2665*
Coggan, K ... 3443*
Cohen, J ... 1496
Cohen, J M ... 1508*, 2143, 2989
Cohen, L ... 2129
Cohen, M ... 2129, 2247
Cohen, M D ... 1731*
Cohen, S M ... 1254, 1787*, 3184

Cohen-Kaminsky, S ... 1293
Cohn, B ... 2730
Coker, E S ... 1452*
Coksari, G ... 1485
Colagiovanni, D ... 3262*
Colby, S M ... 2216*
Cole, A P ... 1199
Cole, E ... 3501
Cole, S L ... 1367*
Cole, T B ... 1169, 1206
Colegate, S ... 1992
Coleman, G D ... 3276
Coleman, J ... 2720
Collado, M S ... 2006
Colli-Dula, R C ... 2953
Collier, A ... 3317b*
Collier, G ... 2645*
Collier, R H ... 2411
Collins, E-M S ... 2403
Collins, J ... 1423, 2774
Collins, L ... 2745
Collins, R ... 1179
Colomina, M T ... 2915*, 2916
Colón-Rodriguez, A ... 1575*
Comba, R W ... 1928
Composto, G M ... 2094
Conboy, L ... 2912
Concha-Lozano, N ... 3403
Condon, M ... 1561
Conklin, D ... 1836, 2134
Conklin, D J ... 1048*, 2250, 3326*
Conley, J M ... 2549*, 2550
Connelly, J ... 1093
Conolly, R ... 1102, 2210*, 2214
Conrad, K ... 1188
Conrad, K A ... 1166
Consevage, K ... 2021*
Constant, S ... 1707*, 3552*
Constantinos, M ... 2032
Contal, S ... 2805
Contreras, P ... 1951
Coogan, T ... 1641
Cook, A ... 2852
Cook, D ... 1461
Cook, E ... 1176*
Cook, R ... 1735
Cook, T J ... 2352*
Cooke, G ... 3028
Cooney, C ... 1196, 3198
Cooper, G ... 2704, 2705
Cooper, G S ... 2707
Cooper, K ... 3554
Cooper, S ... 2212, 2596*
Cooper, T K ... 2874
Copeland, L ... 1065, 1302
Copeman, C ... 1649, 3530
Cora, M ... 2131
Corbett, K ... 1165
Cordes, J ... 2839
Coriano, C ... 2439*
Corke, S ... 3037
Corley, R A ... 2144*, 2215, 2216, 2234, 3043, 3462
Cormier, S A ... 1303, 2245
Coronado, V ... 2096, 2127
Corrales, J ... 2323*, 2324, 3283
Correa-Basurto, J ... 2956
Correia, A ... 3346
Correll, J B ... 3430
Corsini, E ... 2861*
Corteil, G ... 2249
Cortes, L ... 3041
Corton, C ... 1758*
Corton, J C ... 2616, 2624
Corvaro, M ... 2208, 2470*
Coryell, M P ... 1252*
Cory-Slechta, D ... 1167
Cory-Slechta, D A ... 1166, 1168, 1188
Coskun, E ... 1390, 1404, 1417*
Coslo, D M ... 2435
Cosnier, F ... 2791
Costa, C ... 2775
Costa, L G ... 1169, 1206, 1207
Costa, M ... 1562, 2064, 2072, 2073, 2075, 2322, 2894, 3218, 3338
Costa, P H ... 3248*
Costa, V ... 1963
Costantin, J ... 1829
Cota, J ... 2620*
Côté, J ... 1144
Côté, M-H ... 1926
Cotelesage, J ... 1583
Cotovio, J ... 1612, 1613, 1614, 2007, 2182, 2206, 2477
Cottagiri, M ... 1974
Cottrez, F ... 2199
Coull, B ... 1071
Coulter, S J ... 2045
Coumoul, X ... 3427
Courage, C ... 2596
Couroucli, X ... 3143
Couroucli, X I ... 3152, 3154*
Courtney, S ... 2934
Couttet, P ... 3461
Covaro, M ... 2576*
Covington, T ... 2228
Covington, T R ... 2217
Cowan, D ... 2669
Cowan, D M ... 1310, 2695, 2978
Cowan, V ... 2991
Cowan, V E ... 2831*
Cowie, A M ... 2412
Cowie, D ... 2018
Cowles, D H ... 2300*
Cox, K D ... 2138, 2152, 2153*, 2154
Coyle, J ... 2854, 3174
Crabtree, M ... 2327
Craciun, F L ... 3138
Crago, J ... 2955
Craig, E ... 2690*
Crameri, F ... 1644*
Crane, C ... 3016
Crawford, J ... 1638
Crawford, R ... 1364, 1736, 2686, 2687
Crawford, R B ... 1118, 1340, 1341, 1342, 1360
Creek, M R ... 2411, 3447, 3448
Creppy, E E ... 3184
Crespo, N E ... 1905*
Crespo, S M ... 2855
Creutzenberg, O ... 1510*, 2542, 3499
Crews, S ... 2096
Crews, S R ... 2127
Criscoe, D ... 3160*
Crispe, I N ... 1940
Croasdell, A ... 1911*
Crofton, K ... 2606
Crofton, K M ... 1886
Cromie, M ... 1477*
Cronin, M ... 2163, 2476*
Cronin, M T ... 1097, 2475, 2656
Cros, D ... 3184
Crose, L ... 2074
Croteau, P ... 3041
Croutch, C ... 1373
Croutch, C R ... 2110, 2111, 2116, 2121, 2123, 2124*
Crouthamel, B ... 3446
Crow, J A ... 2725
Crowell, S R ... 2234, 3462
Crowl, C ... 3200
Crowley, E ... 2952
Crudu, A ... 1113*, 2015
Cruikshank, W ... 2181
Crump, K S ... 2137
Cruse, C ... 2678
Cruz, M G ... 2901
Cruzan, G ... 2036*, 2038
Cruz-Vazquez, C ... 3021
Cruz-Vazquez, C R ... 2271
Csanaky, I L ... 2316
Cubberley, R ... 2646, 3512
Cuccia, J ... 2058
Cucullo, L ... 1621, 3044
Cuddapah, S ... 2322, 3210*
Cuevas, E ... 2347
Cui, H ... 2906
Cui, J Y ... 2300, 3144, 3270
Cui, X ... 2492
Culbreth, M ... 1580*
Cullen, J M ... 2698*
Cullen, W R ... 1478
Culley, T ... 2110, 2111, 2123*, 2124
Cummings, B ... 3135
Cummings, B S ... 2897, 2908, 3447, 3448
Cummings, C A ... 1609
Cummings, K ... 1314
Cummings, K J ... 1558
Cummings, T ... 1238
Cummings-Lorbetskie, C ... 1274
Cumpston, A ... 3485
Cumpston, A M ... 1199, 1284, 1313
Cumpston, J L ... 3485
Cunningham, C K ... 2799
Cunningham, F H ... 2664*
Cunny, H ... 3261
Cunny, H C ... 3273
Cureaux, S ... 3394
Curley, J L ... 1158*
Curran, C P ... 1181, 1195, 1379*, 1382
Currie, H ... 1922
Currie, R ... 2455
Currier, J M ... 1102*
Curry, S ... 2319, 2491
Curry, S C ... 3178
Custer, L ... 1399
Cutler, S J ... 3282
Cyphert, J M ... 1281
Czich, A ... 2647
Czprynski, C J ... 3151

D

Da Silva, M ... 3259
da Silva, T D ... 2855
Dabadi, M ... 3277
Dach, K ... 1161*
Dachir, S ... 2129*
Dadhania, V P ... 1964*
Dagda, R ... 1266
Dahirel, M ... 3237
Dahl, J E ... 2443, 2444, 2445*
Dahlman, H J ... 1299
Dahm, M ... 3488
Dai, X ... 1831*
Dail, M B ... 1877, 2109*
Dailey, L A ... 3215
Daily, L ... 3220
Dalal, V ... 2190
Dalmas, D ... 2849*
Dalrymple, A ... 3040*
Dalton, C H ... 2101*
Daly, W T ... 3080*, 3388
Damale, P ... 2337
Dambach, D ... 1638
Damodaran, T V ... 2405*
Dancy, B C ... 1270
Dandekar, D S ... 1893
Dandekar, T ... 1116
Dandley, E ... 3490
Dandley, E C ... 3494*, 3497
Dang, V ... 2939
Dang, Z-C ... 2718*
Danilenko, D ... 1638
Danov, O ... 2710*
Dao, T L ... 2095
Dargan, P I ... 3472
Das, K ... 2172, 3439
Das, N ... 2052
Das, S ... 1852, 1902*, 1920, 1949, 2212*, 2596, 3061
Das Banerjee, T ... 1266
Dasari, S ... 1326*
Dasenbrock, C ... 2016
Dash, A ... 2006*
Dasmahapatra, A K ... 1487, 3194*
Daston, G ... 1805, 3384*
Daston, G P ... 1117
Dattaroy, D ... 1852, 1902, 1920, 1949*
Dávalos-Pérez, A ... 3219
Dave, N ... 2736
Davenport, S ... 2551
Davidson, D C ... 1496*, 2783
Davidson, T ... 3440
Davies, D ... 2263
Davis, A P ... 1086
Davis, B A ... 1531*
Davis, C ... 3480
Davis, J ... 1422, 2225
Davis, J W ... 2925
Davis, K J ... 2799
Davis, M ... 3108
Davis, S ... 3107
Davoren, M ... 2128*
Daws, L C ... 3086
Day, B ... 2125
Day, B J ... 1209, 2126, 2406
Day, M ... 1379
Dayton, R D ... 2384
D'Cruz, O J ... 1655*
de Cassia da Silveira e Sa, R ... 3408
De Cesare, M ... 3403
de Conti, A ... 2035, 2084*, 3189
de Gannes, M ... 1737
de Graaff, P ... 2873
de Groot, A ... 1152, 1157
de groot, M W ... 1154*, 1157
de Kok, T M ... 2612
de Korte, T ... 1744*, 1830
De Los Santos, G ... 3095, 3099
De Miranda, B R ... 2377*
de Oliveira, D P ... 1408
de Oliveira Mallia, J ... 2772*
De Raat, K ... 2988
De Ron, P ... 2722
de Sousa, D ... 3511
De Vizcaya-Ruiz, A ... 1216, 1500*, 1906, 2253
Dean, D A ... 2296
Dean, J ... 2164*
Dean, T ... 1791
DeAngelo, A ... 1056
Dear, J W ... 1994, 2727, 3472
Dearfield, K ... 2267
Dearman, R J ... 1939, 1947*, 1948
Dedon, P C ... 2742
Deepe, G S ... 1598
Deering-Rice, C E ... 2244, 2251
DeGeorge, G L ... 2136
Degido, G ... 3535
Degn, L L ... 2765
Degraff-Miller, L ... 3505
Deguchi, J ... 1239, 1979
DeHate, C ... 1684*
DeHate, R ... 1684
Deisenroth, C ... 1867, 2005*, 2018
deKnecht, J ... 2526*
Del Bufalo, A ... 2865*
Del Pino, J ... 1186*, 2393
Del Pozo, J ... 1994
Del Razo, L M ... 1261, 1262, 3130
del Rio, C ... 2848
del Rio, C L ... 3531*
Del Rio, E ... 3265
Delacruz, E ... 2136
DeLaForest, A ... 1832
Delaney, B ... 3060, 3064
Delaney, L ... 3084
Delaney, S ... 1225
Deline, S ... 2452
DellaTorre, M ... 2938*
Deloid, G ... 3331*
DeLoid, G M ... 1508
DeLorme, M ... 2659*
DeLouise, L ... 1503
Delp, J ... 2338*, 2340
DeMarini, D ... 2252, 2266
DeMarini, D M ... 1407, 1448
Demchak, J ... 2605
Demchuk, E ... 2133, 2683*
Demirag, F ... 2748
Demokritou, P ... 1496, 1508, 1700*, 2783, 3328*
den Hartogh, S C ... 1744, 1830
Den Hond, E ... 1142
Deng, A ... 2678
Denison, M ... 3393
Denison, M S ... 2299
Denluck, L ... 2797*
Dennis, K E ... 2339
Dennis, W E ... 1541, 2728
Denslow, N ... 2635
Denslow, N D ... 2939*
Dent, M ... 2174
Dent, T ... 3236
Denton, R ... 1674*
Derk, R ... 1496, 2783
Dertinger, S ... 1060, 1061, 1410*, 2639
Des Marais, T ... 2064
Des Marais, T L ... 2072*
Desai, K ... 3010
Desai, V D ... 3475
DeSantis-Rodrigues, A ... 1600
Desaulniers, D ... 1274*
Deschl, U ... 1820, 1821

Descotes, J ... 1636
Deshmukh, D ... 2070*
Deshmukh, N ... 1459
Detroyer, A ... 2865
Deutsch, D ... 3486
Deveau, M ... 1045*
DeVito, M ... 2179, 2224, 2294
DeVito, M J ... 2604, 3017, 3089*, 3150, 3177, 3528
Devlin, R ... 1071, 2243*
Devlin, R B ... 1429, 3215
Devorak, J ... 1323, 2130
Devosse, R ... 2249
Devoy, J ... 2791
DeWitt, J ... 1843*
DeWitt, J C ... 1842
Dey, I ... 1435
Dezell, S A ... 2092*
Dhadra, S ... 2646
Dhaibar, H A ... 2384
Dhakal, K ... 1191, 2409, 2410
Dharwar, N ... 2212
Di Carlo, M B ... 1235
Di Giulio, R T ... 1848, 2951, 3038, 3284
Di Prima, M A ... 2450, 2453
Di Renzo, F ... 3279, 3280
Dial, S ... 1388
Diamante, G ... 2949*
Dianati, E ... 3390
Dias da Silva, D ... 1443
Diaz, D ... 1638, 1666
Diaz, E A ... 2855
Diaz, J ... 2986
Diaz, J H ... 2964*
Diaz, M J ... 2393
Diaz-Diaz, C J ... 2076*
Diaz-Sanchez, D ... 1071, 2490, 3215, 3220
Dickerson, A ... 2242
Diehl, A M ... 1902, 1920, 1949
Dietert, R R ... 3373*
Dietrich, D R ... 1818*, 3056
DiGiovanni, J ... 2065
Dill, J A ... 1308
Dillingham, C M ... 3413
Dillon, D ... 3040
Dilworth, C ... 1615, 2010, 2847
Dimond, S S ... 1138*
Dinesdurage, H ... 1411
Ding, J ... 2257*
Ding, J M ... 3183*
Ding, L ... 2292
Ding, W ... 2768*
Ding, X ... 1288, 2292, 3147, 3458
Dingemans, M M ... 1152*
Dinis-Oliveira, R ... 1963
Dinu, C ... 3483
Dioquino, C P C ... 2484
Disdier, C ... 2791*
Dishaw, L V ... 1198*
Ditzel, E J ... 3345*
Divi, K V ... 2738
Dixon, L ... 1630
Dizdaroglu, M ... 1390, 1404, 1417
Do, K H ... 1942
Doak, S ... 2772
Dobo, K ... 1391*
Dobrovolsky, V N ... 2592
Dobrzański, T P ... 2024
Dodd, C A ... 1813
Dodd-Butera, T ... 3352*
Dodmane, P R ... 2159*, 3184
Doerge, D R ... 1139, 1140, 2084
Doerr, L ... 1829
Doheny, D L ... 1867
Doherty-Lyons, S ... 1846, 3248
Doi, A M ... 2706
Doi, Y ... 2033
Doig, A ... 1939
Dolan, D ... 2666
Dolan, E ... 1225
Dolde, X ... 1171
Dolin, C E ... 2211
Dolinoy, D ... 1089
Dolinoy, D C ... 1858, 3203, 3204
Dolinsky, P ... 2077
Dolislager, F ... 2691
Doll, M ... 3115
Doll, M A ... 2067, 2496
Domesle, A ... 2267
Dominah, G ... 2391, 2392
Domingo, J L ... 2315*, 2795, 2915, 2916
Domínguez-Pérez, M ... 1973
Domoradzki, J ... 2925
Dong, C ... 3483
Dong, J ... 1251
Dong, M-S ... 2724
Dong, W ... 1483, 1483*, 1809*, 2888, 2944
Dong, X ... 2127
Dong, Y ... 1193, 1194, 3415
Donio, M ... 2852
Donley, E L ... 3299
Donnelly, A ... 2454
Donner, E M ... 2543
Dony, E ... 3487
Dorfmüller, P ... 1293
Dorman, M T ... 1798
Dornbos, P ... 1100, 2686, 2687*
Dorta, D J ... 1127, 1408
Doshi, K A ... 2048*
Doshi, U ... 3145, 3146
Doshna, C ... 3480
DosSantos, G ... 1833
Dosumu, O A ... 1248, 1249, 1547, 1568
Douillet, C ... 3343*
Dourado, D ... 1963
Dourson, M L ... 2992, 3410
Douville, J ... 3530
Dover, E ... 3343
Dover, H ... 1346
Dow, J A ... 1271
Doyle, E ... 2240
Doyle-Eisele, M ... 1114, 2086, 2100, 2261, 2742, 3444, 3535*
Dragan, Y ... 1124
Dragan, Y P ... 3385*
Drager, L ... 1309
Draper, R ... 2796, 3486
Draugelis-Dale, R O ... 2931
Draz, H ... 1481, 2044*
Drechsel, D A ... 2273*
Drega, S ... 1157
Dreher, K ... 2756*
Dreier, D ... 2635*
Dremier, S ... 2722
Dreval, K ... 3189*
Drey, H ... 2829
Dreyfus, C F ... 2370
Driessen, M D ... 1518
Drizik, E ... 3396
Drobna, Z ... 1259, 1261, 1262, 2749*
Drury, S ... 1272, 1550
Dryden, G ... 1901
Du, G ... 2367
Du, K ... 1971, 1981*, 3092
Du, Y ... 1587, 1597
Duags, T R ... 2844
Duan, J ... 1509, 3438
Duan, J D ... 1820, 1821
Duan, L ... 3092*
Duarte, D J ... 1231
Duarte, F V ... 1127
Duarte, J A ... 1963
Duarte-Sustaita, J J ... 1255
Dubé, E M ... 2270
Dubinett, S ... 3396
Dudal, S ... 1644
Duellman, S ... 3553
Duenas, K ... 1230
Duenbostell, A ... 1742, 2857
Duerr, O ... 3536*
Duffney, P F ... 1283, 3047*
Duffy, P ... 2685
Dufva, M ... 1997
Dugas, T R ... 1303, 1439, 3324*
Duistermaat, E ... 2815
Duke, K S ... 3494, 3500, 3502*, 3503
Dulaney, M D ... 2233*
Dulize, R ... 1106, 3036
Dumas, S J ... 1293
Dumont, C ... 1926*
Duncan, K ... 1071
Duniec-Dmuchowski, Z ... 3457
Dunivin, T ... 1346
Dunlap, P E ... 1811, 2294*
Dunlevy, J R ... 1273
Dunnick, J K ... 3177
Dunnick, K ... 1314, 2780
Dunska, M ... 1744, 1830
Duong, H ... 2731
Duplan, H ... 3512
Dupre, T ... 3115
Dupre, T V ... 2496*
Durasi, İ M ... 3205
Durham, D ... 1234
Dusserre, C ... 2431
Dutton, N ... 3170
Dutton, N D ... 3419
Duval, C ... 2430, 3427*
Duverna, R ... 2267
Duydu, Y ... 2813
Dwivedi, A M ... 1282*
Dydak, U ... 1596, 2354*, 2355
Dye, J A ... 1302, 3195
Dykens, J ... 1004*
Dyring Jakobsen, S ... 3242
Dzantor, E K ... 1430
Dzierlenga, A L ... 1985*

E

Eaddy, J S ... 3175, 3181
Easley, C A ... 3247, 3253
Eastwood, J ... 3028
Eaton, A ... 3060
Eaton, D L ... 1105, 2352
Eaton, J S ... 1608*, 1610
Eberle, C S ... 1631*
Ebid, H A ... 2388*
Ebokaiwe, A P ... 3245*
Eckert, P ... 1116, 1449
Eddleston, M ... 2727
Edelman, E R ... 2441, 2442
Edginton, A ... 3321*
Edmondson, D A ... 2354, 2355*
Edoff, K ... 2807
Edrissi, B ... 2742*
Edwards, G ... 3477
Edwards, J ... 1530*, 1634*
Edwards, J A ... 3004, 3005*
Edwards, J R ... 3122
Edwards, M ... 2089
Edwards, M T ... 1335*, 3086
Edwards, P ... 3147
Edwards, S ... 1003*
Edwards, S W ... 1101
Efremenko, A ... 3071
Efremenko, A Y ... 1413*
Egeghy, P ... 1680
Egelberg, P ... 3545
Egnash, L A ... 3299
Egoshi, N ... 3456*
Ehrich, M ... 1659*, 2408
Ehrich, M F ... 1999
Ei, K ... 2193
Eid, A ... 2385*
Eidemiller, B ... 1372
Eilstein, J ... 2865, 3512
Einstein, D R ... 2144, 2216
Ekhator, O ... 2356
Eklund, C ... 2210, 2231
Eklund, K ... 3190
El Amrani, A-I ... 2845
El Amrani-Callens, F ... 2845
El Sayed, K A ... 1491, 3454
Elamin, A ... 3029, 3402
Elango, N ... 1120
El-Ashry, R ... 2387
Elcombe, B ... 2433, 2706
Elcombe, C ... 1442, 2068, 2240, 2433, 2436, 2706
Eldawud, R ... 3483*
Elder, A ... 2539*, 2540
Elend, M ... 3548
El-Fawal, H A ... 1227*, 1585*
Elferink, C ... 2428
Elferink, C J ... 1052*, 2427
El-Gazzar, R M ... 1585
El-ghoneimy, A A ... 1431
Elhalwagy, M E ... 1210
El-Husseiny, M ... 2387
Elias, P D ... 2194
Elihn, K ... 2788
Ellehoj, E ... 2997
Ellinger-Ziegelbauer, H ... 3476
Elliot, D ... 1292
Elliott, J T ... 1522
Ellis-Hutchings, R ... 2063*
Ellison, C M ... 1097
Ellison, L ... 2721
Elmahdy, A ... 3399
El-Mahdy, M A ... 3399*
El-Masri, H ... 2222, 2607, 3320*
El-Mesallamy, H O ... 2825
Elmore, S ... 1353
Elmore, S E ... 2269, 2283*, 3012
Elobeid, M ... 3250*
Eloff, J N ... 1495
Eloff, K ... 1433
Elosegui Rodriguez, P ... 1576*
El-Saied, E ... 3006
Elsass, K E ... 1620*
Elser, B A ... 2042*
ElSohly, M A ... 3282
El-Tawil, O ... 3006*
Ema, M ... 2156
Emami, A ... 1643*
Embry, M ... 2925
Emerick, G L ... 2399
Emery, S ... 2182
Emikpe, B O ... 2954
Emnéus, J ... 1997
Emond, C ... 2227*, 3427
Emond, F ... 3530
Enayah, S H ... 1592*
Endo, M ... 2773
Endo, N ... 1564*
Endo, T ... 1190
Endoh, S ... 3482
Endres, J ... 1457, 1458
Endres, J R ... 1459*
Eng, D ... 2836
Eng, K G ... 3075
Engel, M ... 1060
Engel, S ... 3260*
Engel, S M ... 3274
Engels, K ... 2817
England, J ... 1930, 3107
Englert, N ... 1837
Englert, N A ... 3200*
English, J C ... 2138, 2152*, 2153, 2154, 2155, 3007
Engstrom, A K ... 1593*, 1594
Enoch, S ... 2163
Enoch, S J ... 2656
Enright, H A ... 1153, 2390*
Enriquez Cortina, C ... 1978
Enzenauer, R W ... 2116
Ercal, N ... 2808
Erdei, E ... 1559, 1560*
Erdely, A ... 1289, 1701*, 2782, 3485, 3488, 3496
Erickson, R ... 1638*
Erickson, R P ... 3179
Erickson, S ... 3198
Eriksson, P ... 2394
Erkens, T ... 2723*
Ernst, H ... 2537, 2542, 3499
Ernstgård, L ... 1282, 1944*
Escalon, B L ... 1806
Escamilla-Rivera, V ... 1500
Esch, H ... 1116
Esch, H L ... 1449, 3391*
Escher, C ... 2000
Escher, S ... 2163, 2710
Escher, S E ... 2650, 2684
Escobar-Wilches, D C ... 1141
Escobedo Calvario, O A ... 1978
Escudero-Lourdes, C ... 1256*
Espenschied, S ... 1791
Espey, M G ... 1916
Espin Perez, A ... 2612*
Espinosa-Cotton, M ... 1664
Esposito, E R ... 1315*
Essing, A ... 2293
Esswein, E J ... 1686
Estevan, C ... 3265
Esteves, K ... 1272, 1550
Estevez, A ... 3272*
Estevez, J ... 3265
Estrella, B ... 2069
Ethridge, S E ... 1538*
Eto, K ... 1583
Etter, S ... 1821
Eubig, P ... 1230*

Evangelista, E A ... 3163*
Evanoff, E R ... 1273
Evans, J ... 2240
Evans, M ... 1238
Evans, M V ... 2608*
Evans, N ... 2549
Evers, R ... 2731
Evilia, C ... 2867
Evoy, R ... 2277
Exler, D ... 1658
Eye, T ... 1289, 3485, 3488, 3496
Eyes, T ... 1939*

F

Faber, S C ... 2299*
Fabian, E ... 1115, 2043, 2200, 2223, 2463, 3536
Fabyanic, E ... 1441*, 1922
Fadeel, B ... 2807
Fadel, E ... 1293
Fader, K A ... 1100, 1103, 1118*
Fagbohun, A F ... 2954*
Fahnestock, L A ... 1265*
Fair, M ... 2238
Fajana, O O ... 1535
Falcon-Rodriguez, C I ... 1906*, 2253
Falkner, K C ... 1962, 2733*, 3170
Fallah, S ... 2286*
Famili, A ... 1611
Fan, D ... 1949
Fan, L-W ... 1232, 2345
Fan, M ... 2906
Fan, W ... 3206
Fan, X ... 2292*
Fan, Y ... 1737, 1823
Fang, H ... 1870, 2750*, 3183, 3187
Fang, J-L ... 3455*
Fang, L ... 3201, 3338
Fang, M ... 2888, 3068*
Fang, X ... 1845*
Fang, Y ... 3153
Fantegrossi, W E ... 2347
Farabaugh, C S ... 1394*
Farcas, M T ... 1291, 2803
Faria, E C ... 2682
Faria, M ... 1806
Faris, R ... 3059*
Faris, R A ... 3554
Farland, W ... 3367b*
Farman, C ... 1611
Farombi, E O ... 1817, 1968
Farombi, O E ... 1923
Farraj, A ... 2838
Farraj, A K ... 2252, 2260, 2262*, 2263
Farris, B ... 1289
Farris, B Y ... 1284, 3496
Fasani, R A ... 1084
Fasullo, M ... 1419*
Fasulo, M ... 3157
Fatoki, J O ... 1249
Faulhammer, F ... 3536
Faulk, C ... 1089*, 3203
Faustman, E M ... 1498, 2181
Fausto, N ... 2219*
Fautz, R ... 3548
Favela, K A ... 1682
Favrat, B ... 3403
Favre, N ... 1225
Fay, K ... 2925
Fechner, J H ... 1945*
Fedan, J S ... 1199, 1284*, 1313, 2346, 3489
Fedewa, H C ... 1965
Fedtke, N ... 2988
Fegert, I ... 2068*, 2433, 2706
Feldman, D ... 2105
Felix, S H ... 1153, 2390
Félix-Arellano, E E ... 1260
Fell, L ... 2993
Felten, V ... 2805
Felter, S ... 2677
Felter, S P ... 2475
Felton, R P ... 2799
Felzenszwalb, I ... 2241
Fendorf, S ... 2736
Feng, C ... 1275
Feng, S C ... 1619*
Feng, W ... 1194, 1814, 1901, 1950, 1961, 3415*
Fennell, T R ... 2818, 2819, 3273, 3436, 3453*
Fenner, S ... 1658
Fenner-Crisp, P ... 2478
Fensterheim, R ... 2166
Fenton, M ... 2294
Fenton, S ... 3271
Fenton, S E ... 1856, 1857, 3241, 3389
Ferdosi, H ... 2149, 2983*
Ferency, G ... 1927
Ferguson, P L ... 3495
Ferguson, S ... 2179, 2224, 2297*
Ferguson, S S ... 1451, 2294, 2604
Ferloni, A ... 2901
Fernandes, A S ... 2241
Fernandes, J ... 2360*
Fernandes, L S ... 2399*
Fernandez, C J ... 3409
Fernández, J ... 2775
Fernandez-Metzler, C ... 1790
Feroe, A ... 2631
Feroe, A G ... 2972
Ferraris, E ... 2046*
Ferraz, B ... 3296
Ferraz, E R A ... 2241*
Ferris, E ... 3531
Fersuson, S S ... 3150
Fertig, N ... 1745, 1829
Fessler, M B ... 1064
Fett, D ... 2328
Fetterman, J L ... 3325*
Feychting, K ... 2966
Fiebelkorn, S A ... 2664
Fiedler, R ... 1402
Fiedler, S ... 2077
Fields, P E ... 1934
Fields, W ... 1325
Fields, W R ... 3041
Figar, B S ... 2901
Figler, R ... 2006
Figueiredo, J ... 3107
Figueiredo, M ... 1351
Fikree, H ... 2150, 2151*, 2667
Filgo, A ... 3271
Filgo, A J ... 1856, 3241*
Filgueiras, C C ... 1243
Filho, M S ... 2855
Findley-Van Nostrand, D ... 3347
Fink, D M ... 3266
Finkelstein, J N ... 1290, 1903
Finkelstein, Y ... 2098
Fink-Gremmels, J ... 2873
Finley, B ... 2974
Finley, B L ... 1300, 2139, 2140, 2672
Finnell, R H ... 1245
Fioravanzo, E ... 2163, 2476
Fiorentini, F ... 2740
Fisch, L ... 1513, 2759*
Fischer, N O ... 1153*, 2390
Fishbein, M C ... 1904
Fisher, A ... 1056
Fisher, E ... 3073
Fisher, J ... 2239, 3319*, 3438
Fisher, R L ... 2486
Fisher, S J ... 3141, 3236, 3409
Fitch, M N ... 1304*
Fitschen-Brown, M ... 3351*
Fitz, L ... 2482
Fix, N ... 1314
Flannery, B ... 2275*, 2395
Flaugher, K ... 2415
Flaws, J A ... 1855, 1856, 3224, 3230
Fleck, S C ... 1140*
Fleischer, J ... 2465
Fleming, C R ... 2287*
Fleming, S ... 2356*
Fletcher, B L ... 3273
Flick, B ... 1788*, 2632
Flick, M J ... 1954, 1965
Flockton, H ... 1325
Flor, S ... 1421*
Flores, K M ... 1221*
Flowers, L ... 1436
Floyd, D ... 2849
Fluharty, K L ... 1199
Fluri, D A ... 3460*
Fly, A D ... 1456
Flynn, M R ... 2367
Flynn, N ... 2106
Flynn, T J ... 3049
Fokkens, P H ... 3237
Foley, B ... 1871
Foley, J F ... 1609
Foltz, D ... 2197
Font, G ... 3067
Foos, B ... 2148*, 2717
Foote, S ... 1238
Forbes, K P ... 2492*
Forcelli, P A ... 1247*
Ford, J ... 1605
Ford, K ... 1991
Ford, S ... 1380*
Ford, S M ... 3124
Foreman, J E ... 2648
Formiller, E ... 1573*
Fornace, A ... 1084
Forreryd, A ... 2205
Forshee, R A ... 1642
Forster, R ... 1607, 2841, 2842, 2845, 3105, 3243, 3259, 3269*, 3518
Fort, D ... 3298*
Fort, T ... 3298
Foster, A J ... 1669
Foster, L ... 1409
Foster, P ... 3261
Foster, P M ... 2549, 2550
Foster, W B ... 1668
Foster, W G ... 3221
Foucault, C ... 3530
Fournier, P M ... 1291
Fowkes, A ... 2619*
Fowler, C ... 1077*
Fowler, P ... 2183*
Foy, C E ... 3345
Frame, A M ... 2691
Frame, S R ... 3449
Franchini, A M ... 1362*
Francis, M ... 1910*
Franco, R ... 1024*, 1026*
Franco-Bernardes, M F ... 1408
Frank, E A ... 1296*
Frankel, S ... 1247
Franken, C ... 1142
Franklin, P ... 3090, 3099
Franks, D G ... 2424
Frantz, A M ... 1927*
Franzen, A ... 2166
Franz-Jonas, B ... 1615
Franzosa, J A ... 1882, 3188*, 3300
Frawley, R ... 1353*
Frazer, D ... 3485
Frazer, D G ... 1199, 2346
Frazier, K ... 1658
Fredriksson, A ... 1236
Fredriksson Puigvert, L ... 2000
Freebern, W ... 1332
Freeborn, D L ... 1215*
Freedland, J ... 1419
Freedman, J H ... 1811
Freeman, E ... 1395, 2692*
Freeman, J L ... 1588, 1792, 1793, 2517*, 2946
Freeman, M ... 3084
Freimoser-Grundschober, A ... 1644
Frejo, M T ... 2393
Freke, M ... 1628*
French, J E ... 2739
French, K ... 1658, 2849
Frentzel, S ... 1106, 3552
Freriks, M ... 3536
Freshman, R D ... 1735
Frey, J ... 3216*
Frey, J S ... 1094
Frey, O ... 3460
Frieboes, H B ... 2211
Friedhofff, L T ... 1208
Friedman, M ... 1422
Fritsche, E ... 1082, 1161, 2505*, 3316*
Fritz, G ... 1397*
Fritz, H A ... 1689
Fritz, J M ... 2087*
Fritz, K ... 2752
Fromenty, B ... 1113
Fronczek, F R ... 1623
Frost, K ... 2996
Fruth, D ... 2043*
Fry, C ... 3529*
Fry, R ... 1259, 1261
Fry, R C ... 1262, 1683, 2634, 2654, 2749, 2977, 3199*, 3211
Fryman, P ... 3541
Fu, H ... 1589
Fu, J ... 1251, 1267
Fu, S ... 1551
Fu, X ... 2363, 2366*
Fu, Y-H A ... 3513
Fu, Z D ... 3144*
Fu, Z-D ... 3171
Fuchs, A ... 3548
Fueta, Y ... 3263, 3264*
Fuhrman, K ... 2408
Fujii, H ... 1957
Fujimoto, W ... 1355
Fujimura, M ... 1570, 1579*, 2289
Fujita, K ... 1113, 3482*
Fujiwara, M ... 3289
Fujiwara, R ... 1957, 3173*
Fukuda, M ... 3482
Fukuda, T ... 1539
Fukunaga, S ... 1092*
Fukushima, S ... 2031
Fukushima, T ... 3136, 3249*
Fukuzaki, K ... 3090, 3095, 3099
Fulford, D E ... 3024
Fuller, A ... 1237*
Funabashi, H ... 1239, 1660, 1979
Fung, E S ... 1310*
Fung, G ... 1619
Furey, T ... 3190
Furlong, H C ... 3221*
Furr, J ... 2549
Furrow, E ... 1271
Furtado, K ... 2035
Furue, M K ... 1824
Furukawa, M ... 1604
Furuta, M ... 1476
Furutani, T ... 2816
Furuya, S ... 3189, 3433, 3434
Fuscoe, J ... 1111, 2746, 3073
Fuscoe, J C ... 2614*, 3475
Fussell, K C ... 1866, 2431*
Fuster, E ... 3265

G

Gabos, S ... 1435
Gabriel, S ... 2440*
Gaca, M ... 3037, 3552
Gaca, M D ... 2053, 2188, 3030, 3031
Gaça, M ... 2175
Gaça, M D ... 3041
Gadagbui, B ... 2694
Gadupudi, G S ... 3420*
Gafar, A ... 1481
Gaffrey, M ... 1509
Gagnaire, F ... 2791
Gagné, R ... 2088
Gagné, S ... 1145*
Gaines, L G ... 2691
Gaitens, J ... 1561
Gajewska, M ... 1299
Galandiuk, S ... 3340
Galanko, J ... 3343
Galbiati, V ... 2861
Galbraith, D ... 2974
Galdanes, K ... 2246, 2254
Galicia, A S ... 3243
Galicia, R ... 2605
Galijatovic-Idrizbegovic, A ... 2731
Gallagher, E ... 2323, 2324
Gallagher, E P ... 2936
Galli, C L ... 2861
Gallo, C J ... 1271
Gallo, M ... 2069
Galloway, L ... 2691*
Galvanho, J P ... 1243
Gam, K ... 2257
Gamboa, G ... 2219
Gamboa da Costa, G ... 3455, 3470
Gammon, D ... 2897, 3447, 3448
Gamse, J ... 1016*
Gan, J ... 2665, 2955
Gandolfi, A J ... 1260
Gandour, R D ... 1659
Ganey, P ... 2008, 2502, 2959
Ganguli, S ... 3504
Gannon, A M ... 3221

The numerals following the author names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the presenter.

Gannon, B M ... 2347
Gant, T W ... 3001
Gao, J ... 2858*
Gao, S ... 1810
Gao, T ... 1810
Gao, W ... 1477
Gao, X ... 1499, 1519, 2366, 2771*
Gao, Y ... 3013
Gao, Z ... 2881
Gaou, I ... 1862
Garantziotis, S ... 3498, 3505
Garavan, F ... 2828
Garbarino, V R ... 3086*
Garbow, J ... 2409
Garcia, A J ... 1808*
Garcia, E ... 2100
Garcia, G R ... 2305*
Garcia, J ... 1963
Garcia, J G ... 2602
Garcia, J M ... 2393
Garcia, T ... 2795
Garcia-Alonso, L I ... 2253*
Garcia-Canton, C ... 3031
Garcia-Espiñeira, M C ... 2904, 2929
García-Hernández, H ... 2325
Garcia-Perez, I ... 1107
García-Ramírez, H A ... 1493*
Garcia-Reyero, N ... 1084, 1806*
Garcia-Sterna, R ... 2650
Garcia-Vargas, G ... 1259
García-Vargas, G ... 1261, 1262*
García-Vargas, G G ... 1255, 2749
García-Zepeda, S P ... 3228, 3229
Gardner, D R ... 1461
Garduño-Balderas, L G ... 2790*
Gareau, K ... 2495
Gargas, M L ... 2232*
Garland, M A ... 2792*
Garlick, R ... 2125
Garner, E ... 2060, 3164
Garrett, S H ... 1257, 1258, 1273, 1531, 3127, 3132
Garry, M R ... 2668*
Garssen, J ... 2873
Garza-Lombo, C E ... 1219*
Garzel, B ... 2301*
Gasiewicz, T A ... 1734, 1837
Gasmi, J ... 1481
Gaspar, O ... 3134
Gaspard, M ... 2077
Gasser, R ... 1645
Gassman, N R ... 1390*
Gate, L ... 2791
Gato, W E ... 1521, 1524, 1880, 1895
Gatti, D ... 2753
Gavett, S ... 1065, 1070
Gavett, S H ... 1281*, 1448
Gawdzik, J C ... 1187*
Gbadegesin, M A ... 1446
GCG, M-A ... 1494
Ge, W ... 1865, 1870
Gearhart, J M ... 1094*, 2119, 2217, 2228, 2734, 2887
Gebel, T ... 2541
Geer, C ... 3443
Gehen, S ... 2470, 3546
Gehen, S C ... 2208*, 2674
Gehlhaus, M ... 1436
Gehrke, H ... 2207
Geier, M C ... 3287*
Geiger, L ... 3532
Geiger, P ... 2076
Gelein, R ... 2539
Gellatly, N ... 2646
Gemzik, B ... 1668, 1676
Gennings, C ... 1432
Genter, M B ... 1598
Gentile, M A ... 1301
Gentry, N ... 3192
Gentry, R ... 2166, 2701*, 3410
Georgantzopoulou, A ... 1511, 2805
George, B ... 2721*
George, G N ... 1583
George, M ... 1829, 3498
Geppert, L N ... 1116
Gerads, R ... 1264
Gerbeix, C ... 3518*
Gerber, D A ... 1996
Gerberick, G F ... 2195*, 2466
Gerde, P ... 2171
Gerecke, D ... 2112
Gerecke, D R ... 1600, 2094
Gerlach, C V ... 3138*
Germolec, D ... 1308, 1353, 1451
Germolec, D R ... 2864
Gerrish, K E ... 1609
Gerson, R J ... 1208
Gerstel, D ... 3512
Gerstenfeld, L ... 1883
Gesswein, G ... 3085
Gesumaria, L ... 3218
Gettmann, K C ... 2236*, 2675
Gez, R ... 2129
Ghaisas, S ... 2375*
Ghanem, C I ... 1235*, 3431
Ghibelli, L ... 2807
Ghio, A J ... 1069
Ghodssi, A ... 2618
Ghorab, M A ... 2898*
Ghose, R ... 3452, 3465
Ghosh, R ... 2910*
Ghosh, S ... 1113
Gi, M ... 1092, 2031
Giamberini, L ... 1511
Giambérini, L ... 2805
Giannotti, E ... 2740*
Gibbons, C ... 1044*
Gibbons, J A ... 2107*, 2117
Gibbs-Flournoy, E A ... 1302*
Gibson, C J ... 2371
Gibson-Corley, K N ... 2793, 3425
Giersiefer, S ... 1161
Gilbert, J ... 2495, 3437
Gilbert, K ... 1369, 3198*
Gilbert, M ... 2222, 2504*
Gilbert, S ... 2162
Gilbreath, E ... 1226, 3346
Gill, P ... 1110
Gill, P S ... 2489*
Gill, R ... 1349
Gill, S ... 1569
Gillera, S E A ... 1856*
Gillespie, M E ... 3349*
Gilliam, B ... 3529
Gilmer, J ... 2867, 2868*
Gilmore, R G ... 1893
Gilmour, I ... 2838
Gilmour, I M ... 3195
Gilmour, M ... 1065
Gilmour, M I ... 1448, 2266
Ginsberg, G ... 2717, 3371*
Ginsberg, G L ... 3405*
Gioieli, L ... 2035
Girer, N G ... 2423*
Giri, A ... 2052*
Giri, A K ... 3340
Giroud, C ... 3403
Gitschier, H ... 1988, 2009*
Gitschier, H J ... 1990
Giustarini, G ... 1334*
Giusti, A M ... 1644
Glaab, W ... 1005*, 1677
Glaab, W E ... 2731
Glaros, T ... 2117
Glasscock, A E ... 2384
Glavin, S ... 2212, 2596
Glávits, R ... 1457, 1458, 1459
Glaza, S ... 3090, 3095, 3099
Glazer, L ... 2943
Glendinning, J ... 1750*
Glenn, B S ... 2707
Glenn, T ... 3135
Glenn, T C ... 3018
Glez-Weller, D ... 2278
Glick, A B ... 1733
Gliga, A R ... 2807*
Glineur, S ... 2722
Glisic, B ... 2306, 2945*
Glissmann, S M ... 1093
Glista-Baker, E E ... 3503
Gloekler, L ... 2669*
Glover, K P ... 1124*
Glynn, M E ... 1300, 1690, 2140*
Go, K ... 3169*
Go, Y-M ... 1125*, 1529, 2326, 2360, 3537
Goad, J T ... 2473
Godfrey Robinson, V ... 3273
Godin, C S ... 3085*
Godin-Ethier, J ... 1418
Godleski, J J ... 2855
Goebel, C ... 2197, 2466*
Goedken, M ... 2118
Goel, S ... 1637
Goeminne, N ... 2723
Goerdetier, D ... 1106
Goering, P ... 2719
Goering, P L ... 1776*, 2449, 2450, 2451, 2453, 2762, 2764, 3335
Goettel, M ... 2433, 2706
Gogineni, V ... 3504
Goh, J-Y ... 1630
Gohlke, J M ... 1816, 2967
Goineau, S ... 1317*
Gokulan, K ... 1514, 3026
Gold, A ... 3434
Goldbaum, J ... 3443
Goldberg, A ... 1481, 2044
Golden, T ... 2327*, 2328
Goldsmith, M-R ... 2238
Goldsmith, W T ... 1199, 2346
Goldstein, B ... 1055*
Goldstone, J ... 2122
Goldstone, J V ... 1799, 3159*
Gollapudi, B ... 1395
Golovko, A E ... 2026
Gombar, V K ... 1099*
Gomes, C ... 1113, 2865
Gomes, J ... 1268*
Gomes, K ... 1155
Gomez, G ... 2393
Gomez, M ... 2721
Gómez, M ... 2795*
Gómez Quiroz, L E ... 1973, 1978
Gomez-Acevedo, H ... 1874
Gomis-Tena, J ... 2647
Gondo, Y ... 1389
Gong, B ... 1072, 3166
Gong, L ... 1652*, 1657
Goniewicz, M L ... 3397
Gonnerman, G ... 1796
Gonsebatt, M E ... 1219
Gonzales, C ... 1544
Gonzales, K K ... 2353*
Gonzalez, C ... 2347
Gonzalez, E ... 2453
Gonzalez, E A ... 1174*, 1850
Gonzalez, F ... 1987, 2021, 3161, 3308*
Gonzalez, F J ... 2020, 2024, 3430
Gonzalez, R ... 1677
Gonzalez Suarez, I ... 1106*
Gonzalez-Barranco, P ... 2269, 2283
Gonzalez-Cuyar, L F ... 2352
Gonzalez-Figueroa, E D ... 2256
González-Horta, C ... 1261, 1262
González-Horta, M D C ... 1493, 3130, 3491
Gonzalez-Hunt, C P ... 2369*
Goodale, B C ... 1253*
Gooderham, N J ... 2034
Goodfellow, F T ... 1159*
Goodman, J ... 2673
Goodman, J E ... 2141, 2143, 2992
Goodrich, J M ... 3204*
Goodwin, M R ... 2170
Gopalakrishnan, G ... 1637
Gorczyca, L ... 3232*
Gordon, C ... 3243, 3269
Gordon, C J ... 1068*
Gordon, J ... 1673, 3544
Gordon, M ... 2112
Gordon, M K ... 1600*
Gordon, T ... 1165, 1687, 2246, 2254
Gordon, W ... 3426
Gorelick, D ... 1014b*
Gorham, J M ... 1505
Gormley, M J ... 3236, 3409
Gorniak, S L ... 3463*
Górniak, S L ... 1853
Gosink, M ... 1087*
Gosling, J P ... 2646
Gosse, J A ... 1798, 2332
Gossmann, M ... 2857
Goswami, D G ... 2115*, 2116, 2121, 2126
Gotardo, A T ... 1853*
Goto, M ... 3102
Gotti, A ... 1698, 2472
Goud, N S ... 2447, 3336*
Gould, G G ... 1335, 3086
Gould, J ... 2629, 3440*
Gourzones, C ... 2483
Gouveia, A C ... 2248
Govarts, E ... 1142
Govindarajan, A ... 2122*
Gow, A ... 1297, 2327, 2328
Gow, A J ... 1915, 3350
Gowda, K ... 1733
Gowdy, K M ... 1064*, 3507
Goyak, K O ... 2468*
Goyal, V ... 1617*
Grabovska, O ... 1454
Grace, M R ... 3199
Graham, A ... 3269
Graham, J ... 3440
Graham, J L ... 1850
Graham, O ... 2034*
Graham, S J ... 2101
Graham, U M ... 2540*
Grand, N ... 3524
Grandela, C ... 1744, 1830
Grandidier, M H ... 1612, 1613*, 1614
Grandolfo, D ... 3552
Granitzny, A ... 2016
Grant, K ... 3446
Grant, K S ... 3278*
Granum, B ... 3258
Gray, A ... 2727
Gray, J P ... 1875, 1876
Gray, L E ... 2549, 2550
Gray, W ... 1372, 2059
Graziano, J H ... 3344
Greeley, M A ... 3276
Green, A ... 2069
Green, A J ... 1850*
Green, B T ... 1461
Green, O P ... 2454*
Green, S ... 1442, 1881
Green, S M ... 1445, 1693, 2469*
Greenamyre, J ... 2377
Greenberg, G I ... 2679
Greene, T ... 2166*, 2701
Greenhaw, J ... 1110
Greenhaw, J J ... 1318
Greenwood, J A ... 1804
Gregoire, C ... 3547
Gregoire, S ... 3512
Gregory, H ... 1293*
Grenet, D ... 3065
Grier, A ... 1569
Gries, W ... 1138
Griffith, W ... 2181
Griffith, W C ... 1498
Griffitt, R J ... 3421
Griggs, J ... 3034
Grigsby, C C ... 2228
Grimm, F ... 2711
Grimm, H P ... 1644
Grimm, M ... 3216
Grimm, M D ... 1205
Grisolia, C ... 3296
Grodzki, A-C ... 2416
Grogg, M W ... 3504*
Grollers, M ... 2815
Gromadzka-Ostrowska, J ... 1299
Grondin, C J ... 1086
Gronewold, C ... 3548
Groothuis, G M ... 1997
Grosdidier, E ... 1607*
Grosell, M ... 2934
Grosenbach, D ... 2108
Gröters, S ... 2537
Groux, H ... 2199*
Grove, A ... 1908
Grove, K ... 2452*
Groves, A M ... 1290, 1903*
Groves, R ... 2923
Gruber, P ... 2451
Grulke, C ... 1680, 1682, 2238, 2627
Grulke, C M ... 3539
Grummt, T ... 2860
Grundy, C ... 2003*
Gruner, H ... 1899
Grunig, G ... 1847
Grushka, M ... 1747*
Grusie, T ... 2831, 2991*

Gu, A ... 1863, 2755*, 2804, 2883
Gu, J ... 2853*
Gu, J K ... 3485
Gu, Q ... 2334*, 2335
Gu, S ... 1546
Guariglia, S R ... 2310
Gucer, P ... 1561
Guedes de Pinho, P ... 1967
Guedj, E ... 1106, 3029, 3036, 3401, 3402
Gueguen, Y ... 2688*
Guergues, J ... 1223
Guerre-Millo, M ... 3427
Guerrero-Cazares, H ... 1148
Guguen-Guillouzo, C ... 1970
Guha, N ... 2049
Guignard, C ... 2805
Guignet, M ... 2410*
Guignon, E F ... 3551
Guilarte, T ... 2353
Guilarte, T R ... 1590, 1591, 2310
Guillon, J-M ... 2647
Guillouzo, A ... 1970
Guina, T ... 3087
Guinot, M ... 3237
Guirard, G ... 2652*, 3394
Guleria, K ... 3227
Gullick, D ... 2897*
Gundert-Remy, U ... 3081
Gunn, M D ... 1301
Gunness, P ... 1995*
Guo, C ... 1297
Guo, G ... 1910, 1953
Guo, G L ... 3240, 3350*
Guo, J ... 2963, 3051
Guo, L ... 1312, 2012*, 2592, 2747, 3098*, 3450
Guo, T L ... 1897
Guo, X ... 1228, 1388*, 2592, 2969
Guo, Y ... 2746, 2747
Guo, Z ... 1516*, 1656, 3423
Gupta, A ... 1308
Gupta, I R ... 2757*
Gupta, R C ... 2473*, 2882*
Gurleyuk, H ... 1264
Gusenleitner, D ... 1799
Gustafsson, J-k ... 1794, 1807
Gut, C ... 3216
Gut, C P ... 1205
Gutbier, S ... 2338
Gutherz, S B ... 1247
Gutierrez, A ... 2368
Gutierrez Ruiz, M C ... 1973*, 1978*
Gutierrez-Fernandez, A ... 2278
Gutiérrez-Torres, D ... 1261, 1262
Gutkin, D W ... 1291, 2803
Gutleb, A C ... 1511, 2805*
Gütlein, M ... 3081
Gutman, H ... 2129
Gutteridge, C ... 3042
Guyton, K Z ... 2049*
Guzmán-Mendoza, J J ... 3491*
Gwaltney, S ... 1336
Gwavava, P ... 1320
Gwinn, M ... 1372
Gwinn, M R ... 1777*, 2087
Gwinn, W M ... 1308*
Gyselaers, W ... 1854

H

Ha, M ... 3217
Haase, A ... 1518*
Haase, C ... 2223*
Haber, L ... 2478
Haber, L T ... 3410*
Haberzettl, P ... 1836, 2134, 2250*
Habib, T ... 2228
Hack, C E ... 2217*
Hackbarth, A ... 3499
Hackley, V A ... 1505
Haddad, S ... 2229, 2716
Hadi, M ... 2544
Haedo, R ... 1829
Haenen, G R ... 3015
Hafey, M J ... 2731
Hagen, L ... 3277
Hagey, L R ... 1966
Haggard, D E ... 1802*
Haggerty, H ... 3440
Haghighat, N ... 2669
Hagiwara, A ... 2031
Hagiwara, S ... 3521
Hagstrom, D ... 2403*
Hahn, M E ... 2424*
Hahn, R ... 2112
Hahn, R A ... 1600
Hahne, F ... 2605
Haighton, L A ... 2150, 2151, 2286, 2667*
Hailer, K ... 1269*
Hailer, M K ... 1507
Haines, C ... 2436
Haire, K ... 3174*
Haishima, Y ... 3547
Hajela, R K ... 1575
Hakamata, S ... 1202
Halappanavar, S ... 1291
Halden, R U ... 2939
Halder, M ... 2925
Hales, B ... 1017*, 3390
Hall, B J ... 1164*
Hall, D ... 1150
Hall, J ... 3162
Hall, L ... 2113, 3476
Hall, M ... 1635
Hall, M L ... 1924
Hall, S M ... 2045*
Hallberg, L M ... 3039, 3155
Halley, S ... 2329
Hallgren, S ... 2394
Hallinger, D R ... 1887, 1888, 1889*
Halvorson, K L ... 2186
Hamberg, P ... 2394
Hamdy, N M ... 2825
Hamilton, E G ... 3141*, 3236*
Hamilton, R F ... 1523
Hamilton, T ... 2130
Hamlin, R ... 2848
Hamlin, R L ... 3531
Hamm, G ... 1983
Hamm, J ... 2651
Hammad, S ... 3460
Hammond, S L ... 2337*, 2378
Hampton, J ... 1959
Hampton, M ... 3209
Hampton, S ... 1195
Han, A ... 1441, 1922
Han, C ... 1641
Han, J ... 1810, 3179
Han, T ... 3073, 3475*
Han, X ... 1183, 1241, 1589, 1955, 2925, 3072
Han, Y ... 1460
Hanafusa, H ... 3136
Hanamura, K ... 1172
Handakas, E ... 2472
Haney, J T ... 1536*, 2994
Hanig, J ... 2335
Hanig, J P ... 1840
Hankinson, O ... 3309*
Hanks, C ... 3084
Hanley, A V ... 2720
Hanley, G A ... 2720
Hanna, S K ... 1522*
Hannas, B R ... 1869*, 2549, 2893
Hanneman, W H ... 1917
Hannon, H ... 1573
Hano, H ... 3133
Hansen, D ... 3073
Hansen, T ... 1510, 2016*, 2542, 2710, 3548
Hanson, L ... 2840
Hanson, R ... 2867*, 2868
Hanumegowda, U ... 1676, 2726, 3091
Hao, L ... 1125, 2360
Harada, Y ... 1393, 3522
Harand, K ... 2854*, 3174
Haranosono, Y ... 2603*
Harbeitner, R ... 1799
Harbison, R ... 1684, 2663, 2854, 2999, 3000, 3174
Hardej, D ... 1380, 1381*, 1556, 2895
Hardesty, J E ... 1962*
Hardiman, G ... 2934
Hardin, B ... 2696
Hardisson, A ... 2278
Hardisty, J F ... 2714
Hardval, R ... 3024
Hardwick, R N ... 1959*, 2003
Hargraves, T L ... 2487*
Hari Singh, N ... 1998*, 3557
Harirforoosh, S ... 2720*
Harischandra, D ... 2350*
Harischandra, D S ... 1751b*, 2351
Harishchandra, D S ... 2375
Harkema, J ... 1063
Harkema, J R ... 1100
Harmon, A C ... 1303*
Harney, J ... 2481
Harper, S ... 2797, 3486
Harper, T ... 3246
Harper, T A ... 2079*
Harrill, A ... 1677, 2545, 2753
Harrill, A H ... 3476
Harrill, J ... 1736, 2422*
Harrington, A ... 2246
Harrington, A D ... 2254*
Harris, G ... 2340*
Harris, K L ... 2040*
Harris, M A ... 2237, 2697
Harris, S ... 2750
Harrison, M T ... 1626
Harrouk, W ... 3073
Harstad, E ... 1638
Hart, Z ... 1382
Hartle, J ... 2457
Hartman, J ... 3155*
Hartmann, E ... 3104
Hartson, S ... 2106
Hartung, T ... 1084, 2185, 2338, 2340, 2529*, 2626, 2632, 3408
Hartwell, J ... 1695
Harvey, K ... 1188
Harvey, R B ... 3012
Harvilchuck, J ... 2104*
Harvilchuck, J A ... 2089
Hasan, M ... 2871*, 2872
Hasegawa, M ... 3522
Hasegawa-Baba, Y ... 2028
Hashimoto, T ... 3182
Hashizume, T ... 1393
Hashmi, H N ... 2332
Hashsham, S ... 3351
Hassen, W ... 2483*
Hassoun, E ... 2312*
Haswell, L E ... 2175, 3037, 3041*, 3552
Hatayama, T ... 1355, 1357
Hattori, Y ... 1868, 3267*
Hatzakis, E ... 3430
Haugstetter, J ... 1669
Hauptstein, R ... 1116, 1449
Häußler, S ... 1941
Hautenhauer, G ... 1945
Havel, P J ... 1850
Havener, T M ... 2751
Hawk, M A ... 1622*
Hawkins, B ... 2147*, 2164
Hawkins, B C ... 1320
Haws, L C ... 1371*, 2237, 2697, 3407, 3445
Hayakawa, K ... 1193, 1197, 1892
Hayakawa, K A ... 1884*
Hayashi, H ... 1355, 1358
Hayashi, M ... 2063
Hayashi, R ... 3432
Hayashida, A ... 2810, 2816
Hayden, B K ... 1308
Hayden, P ... 3066, 3069, 3070, 3516
Hayden, P J ... 3396*
Hayes, A W ... 1106, 3029, 3035, 3401
Hayes, M D ... 1947
Hayes, W ... 3400, 3402
Hayes, W A ... 3036
Haykal-Coates, N ... 2262, 2263, 2838
Hays, M ... 1302, 2266
Hays, S M ... 2237
Hazard, E ... 2934
Hazari, M ... 2838, 3195
Hazari, M S ... 1047*, 2252, 2260, 2262, 2263
He, K ... 1162
He, P ... 2969
He, W ... 2758
He, X ... 1656
He, Y ... 1376
Head, A ... 1654
Heart, E ... 1875, 1876*
Hebert, V Y ... 1303, 1439, 2844
Heck, D E ... 2094, 2097, 2099*
Hector, E ... 3204
Hedberg, J ... 2788
Hedge, J M ... 1795*
Hedlin, A ... 2171
Hedrick, E ... 2066*, 2074*, 2418
Heggland, S J ... 3048*
Heidmann, J ... 1743
Heidor, R ... 2035
Heikkinen, P ... 1060
Heilman, J ... 2288*
Hein, D W ... 2067
Heindel, N D ... 2113
Heine, L ... 1114, 2261
Heinonen, T ... 2176*, 3062*
Heinrich, U ... 3499
Heinz, S ... 1980, 3543
Heit, C ... 2752*
Helal, M G ... 3399
Helbing, C ... 1782*
Heldreth, B ... 2476, 2709
Helena, H ... 1148
Hellack, B ... 1518
Helleday, T ... 1417
Hemann, C ... 3399
Hemmann, U ... 1060
Hemmerich, J ... 1788
Hemmingsen, M ... 1997
Hemry, E C ... 1734
Henderson, C E ... 2407
Henderson, K ... 2856*, 3216
Henderson, N C ... 3472
Hendriksen, P J ... 1480
Hendry, D ... 1635
Hendry-Hofer, T ... 2125
Hendy, O ... 2401, 2402, 2902
Hengstler, J G ... 3460
Hennan, J ... 1674
Hennen, J ... 2863*
Hennicke, T ... 1397
Hennig, B ... 1455, 2834, 3417
Hennig, G ... 3096
Hennings, L ... 2025
Henningsen, G ... 2145
Henriquez, A ... 2135
Henriquez, J E ... 1340*, 1341
Henry, E ... 1837
Hentges, K ... 1844
Hentges, S G ... 1138
Hentschel, S ... 2787*
Herbert, G ... 1114, 1309, 2261
Herbert, R ... 1353, 2131
Herckes, P ... 1702
Herman, D A ... 2265*
Hermsmeier, M ... 3514
Hernandes, L C ... 1416*
Hernandez, M ... 2246*
Hernandez, M-C ... 1645
Hernández Cerón, R ... 1261, 1262
Hernández Pando, R ... 1978
Hernández-Alcaraz, C ... 1260
Hernández-Castaños, M R ... 1493
Hernández-Ochoa, I ... 3223, 3228, 3229
Hernández-Ramírez, R U ... 1260
Herner, J ... 1423
Herold, M ... 3536
Herpers, B ... 2595
Herr, D W ... 1215
Herrada, J ... 1625
Herrera, L ... 3535
Herrera-Garcia, G ... 3240
Herring, A H ... 2367
Herrmann, A ... 1606
Herrmann, J E ... 2486
Herron, J ... 1178*
Herry, L ... 2743
Hershberger, P A ... 3397
Hess, S T ... 2332
Hester, S D ... 1056
Heuer, H ... 1161
Heussner, A H ... 1818, 3056*
Hewitt, J ... 3087
Hewitt, N ... 3512
Hewitt, P ... 1222*
Heyer, W-D ... 1387
Heyob, K M ... 1913

The numerals following the author names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the presenter.

Heyward, S ... 2301
Hibatallah, J ... 1616
Hibi, D ... 2050*
Hickman, J J ... 1228, 2007, 2477, 2851*
Hiemisch, A ... 3484
Hiemstra, S ... 1670, 2002, 2595*
Hierlemann, A ... 3460
Higgins, L ... 1442*
Higuchi, M ... 1448, 2263, 2266
Higuchi, M A ... 1429*
Hilberer, A M ... 2175
Hill, M T ... 3222
Hillegass, J ... 2629, 3440
Hilton, G M ... 3490*
Himeno, S ... 1263
Himmel, M ... 2902*
Himmelfarb, J ... 3119, 3123
Himmelstein, M W ... 3449*
Hinckley, J ... 1659, 2408
Hinderliter, P M ... 1694*
Hines, R N ... 1001*, 1039*, 3215
Hines, S E ... 1561*
Hinton, D E ... 1483, 1809, 2888, 2944
Hinton, H ... 1644
Hinton, Z W ... 1842
Hirabayashi, Y ... 1838*
Hiraga, T ... 2888
Hirashima, R ... 3173
Hirata, T ... 1271, 1467, 2598*, 2806
Hirata-Koizumi, M ... 2156
Hirka, G ... 1457, 1458, 1459
Hirokawa, H ... 2403
Hirose, A ... 2156
Hirose, R ... 2295*
Hirota, M ... 2204
Hirsch, S ... 2137
Hirvonen, M-R ... 1851
Hitchcock, K ... 2974
Hitchins, V ... 2453
Hitomi, M ... 1868
Hixon, M L ... 2270
Hixson, C ... 3477
Hnatyshyn, S ... 1674
Ho, J ... 3400
Ho, M ... 2286
Ho, P ... 2352
Ho, T ... 3153
Hoagland, K M ... 2832
Hoard-Fruchey, H ... 2107, 2130
Hobbs, C ... 1422
Hobson, B ... 2409*, 2410
Hocevar, B A ... 3414
Hockenbury, J ... 1382
Hoekstra, J G ... 2352
Hoenerhoff, M J ... 1609
Hoeng, J ... 1106, 3029, 3035, 3036, 3400, 3401, 3402, 3552
Hoffman, G ... 2459
Hoffman, J ... 2834, 3417
Hoffmann, A ... 2026, 2119, 2734, 2887*
Hoffmann, S ... 2632
Hoffmann, V ... 2272
Hoffmann-Doerr, S ... 2684
Hofman-Huether, H ... 2207*
Hofmann, J ... 2085
Hogan, K ... 1436
Hogberg, H ... 2185, 2340
Hogberg, H T ... 3408*
Hok, S ... 1153
Holden, E ... 3545*
Holden, J ... 2356
Holian, A ... 1507, 1513, 1523, 2800, 3501
Holland, E B ... 1194*
Holland, N A ... 2818, 2819*
Hollander, D ... 2663
Hollander, J M ... 2817
Hollanders, K ... 1614
Hollenbach, S ... 3443
Hollnagel, H M ... 2475*
Holman, N S ... 3175, 3181*
Holme, J A ... 1450
Holmes, H ... 2259
Holmes, W ... 2125
Holowiecki, A ... 3295
Holt, L ... 2617
Holzgrefe, H ... 1646*, 2832
Homer, N Z ... 2727
Honarvar, N ... 2200, 2433, 2706
Honda, K ... 3482
Hondebrink, L ... 1157*
Honeycutt, M E ... 2992, 2994
Hong, C ... 1584
Hong, H ... 1079, 1865, 1870*
Hong, S ... 2181
Hong, T ... 2169
Hong, Y ... 3059
Honma, M ... 1389
Hood, D B ... 1213*, 1946
Hooda, P ... 2924
Hook, B ... 3553
Hoopes, M ... 1822
Hooth, M ... 1353
Hoover, J ... 1132, 1560, 2981
Hopf, N B ... 3515*
Hopkins, B K ... 2931
Hopkins, T M ... 1598
Hopper, D ... 1627*
Horii, I ... 3249
Horman, B ... 1856
Horning, K J ... 2364*
Hornung, M W ... 1886
Horsmon, M ... 3103*
Horst, E v d ... 1930
Horton, C L ... 1270
Horton, L ... 2247
Horwath, M C ... 1598
Horwitz, V ... 2129
Horzmann, K A ... 1793*
Hose, D ... 2483
Hosea, B ... 1137
Hosking, S ... 2832
Hosohama, L ... 3192
Hossain, K ... 1263
Hossain, M M ... 2371, 2413*
Hossain, M S ... 2127
Hotchkiss, A ... 2704, 2705
Hotchkiss, A K ... 2713
Hotchkiss, J A ... 1298*, 1704*
Hotchkiss, J J ... 2674
Hottor, H ... 1307, 2968
Hou, Y ... 1251, 1267
Houchens, C ... 3087
Houck, K ... 1151, 1783b*, 1803*, 2640, 2928, 3017
Houck, K A ... 1882, 1886, 3188
Hougaard, K S ... 1774*
Hougaard Bennekou, S ... 3312b*
Houghtling, R ... 3374*
Howard, B E ... 1074*, 1090
Howard, P ... 2750, 2774
Howarth, F C ... 1896
Howell, B ... 2218
Howell, G E ... 2899, 3426*
Hoyte, C ... 2522*
Hozumi, N ... 3263
Hrubik, J ... 2306*, 2945
Hsia, F K ... 2447*
Hsieh, H ... 1598*
Hsieh, J-H ... 1095*, 1451, 2224, 2550
Hsu, E L ... 1735*
Hsu, F-L ... 2117
Hsu, H-W ... 1586
Hsu, W K ... 1735
Hu, C ... 3168
Hu, C-y ... 2986
Hu, H ... 1545, 3204
Hu, J ... 2925
Hu, Q ... 1589*, 1842, 1843
Hu, W ... 2730
Hu, X ... 1589, 2077, 3425
Hua, M ... 2638*
Huang, B ... 2605, 3071*
Huang, B T ... 1316
Huang, B-w ... 2596
Huang, C ... 2060, 3164
Huang, D Y ... 1435*
Huang, E ... 2843
Huang, G ... 1652, 1897*
Huang, J ... 2125, 2406
Huang, M ... 2879*, 3343
Huang, P ... 3075
Huang, R ... 1095, 1811, 2462, 2626, 2642, 3017
Huang, S ... 1347*, 3552
Huang, S Y ... 3514
Huang, S-M ... 2301
Huang, X ... 2367
Huang, Y ... 2370
Huang, Y-C ... 2383
Huang, Z ... 1482
Huaux, F ... 2249*
Hubbard, T D ... 2419*
Hubbs, A F ... 1199*
Huber, C ... 3128
Hübner, J ... 2187
Hudson, L G ... 1275, 1352
Hudson, S V ... 1984, 2211*
Hudzik, T ... 1605
Huebenthal, U ... 1161
Huerta, M ... 2342*
Huestis, J ... 1275*
Hueza, I M ... 1339*
Hughes, B J ... 1298, 2478*
Hughes, M F ... 3406, 3436*
Hughes, M K ... 2846*
Huh, D ... 1709*
Hui, X ... 3509*, 3510
Hulet, S W ... 2824*
Hum, N ... 3428
Humbert, M ... 1293
Hummel, T ... 1748*
Humphrey, G ... 1270
Hunt, L ... 2337
Hunter, D ... 1791
Hunter, D A ... 1521*, 1524, 1895
Hunter, D L ... 1795, 2252
Hunter, E S ... 1822
Hunter, S ... 2547*, 3300
Huppelschoten, S ... 1670, 2293*
Hurley, B ... 3060
Hurley, J ... 1049*
Hurst, S ... 1238
Hurtado, S B ... 1394
Hurtt, M E ... 3274*
Husar, E ... 1645
Hussain, S ... 3327*, 3498*, 3505
Hussain, S M ... 1506, 3504
Hussin, H F ... 1490, 1491*
Huszar, D ... 2048
Hutchinson, J ... 1702
Hutchison, J E ... 2792
Hutsell, B ... 1213
Huttala, O ... 2176
Hutzler, C ... 3525
Hwang, G-W ... 1564, 1566*
Hwang, L ... 1655
Hwang, W-T ... 2047
Hwang, Y P ... 1472
Hyde, J ... 1930
Hynes, J ... 1676, 1828*
Hypes, E ... 2238

I

Iacono, G ... 3530*
Iatropoulos, M J ... 1820
Ibla, J ... 1183
Ibor, O R ... 3297
Ibouraadaten, S ... 2249
Ibrahim, M A ... 1487
Ibrahim, N M ... 2914
Ichihara, G ... 2060*, 2810*, 2816, 3164
Ichihara, S ... 2060, 2810, 2816*, 3164
Ichimura, R ... 1960
Igarashi, I ... 1202
Igbo, J ... 3248
Igbo, J K ... 3424*
Iguchi, T ... 3186
Ihrie, M D ... 3500*, 3502
Ijomone, M O ... 3167
Ikeda, K ... 1660
Ikezumi, M ... 2198
Ikushiro, S-i ... 3456
Ilias, M ... 1487
Imai, N ... 2031, 2198
Imam, M S ... 2799
Imam, S Z ... 2347
Imanirad, P ... 2026
Impinen, A ... 3258*
Inaba, K ... 1570
Inamura, M ... 3148
Infante-Ramírez, R ... 1493
Ingelman-Sundberg, M ... 2000
Ingerson, A ... 3084
Ingle, B L ... 3437
Ingle, T ... 2758
Inoue, K ... 1960
Inoue, R ... 3102
Inselman, A ... 3073
Ippolito, D L ... 2728, 3140, 3468, 3527*
Irfan, M-A ... 2544
Irijalba, I ... 3299
Irrechukwu, O ... 1073, 3061*
Irshad, H ... 2258, 3395*
Irvin-Barnwell, E ... 2970
Isaac, K ... 1681
Isaacs, K ... 1680, 1682, 2222, 2608
Isaacs, K K ... 1679*, 3539
Isama, K ... 2776
Iscan, G S ... 1485*
Iscan, M ... 2748*
Ishibashi, Y ... 1567
Ishida, M ... 1261, 1262
Ishida, Y ... 1566
Ishihara, Y ... 2919*, 2920, 2921
Ishii, Y ... 1868, 2050, 3267, 3456
Ishizuka, Y ... 1172
Iskandar, A R ... 3036*
Islam, S ... 1277
Ismail, A ... 2401, 2902
Ismail, A A ... 2402*
Ismail, R S ... 3399
Isoherranen, N ... 3446
Isse, T ... 2965
Itagaki, H ... 2203, 2313, 2471, 2732, 3055, 3521
Ito, K ... 1604
Itoh, T ... 1957, 3173
Iuliucci, J ... 1650
Iva, N ... 3391
Ivanov, N V ... 1106, 3035, 3036
Iversen, P L ... 2438
Iwata, Y ... 2711*
Iyer, S ... 2030*, 3540
Izquierdo Carerra, X ... 2249
Izumi, M ... 1931
Izuoka, K ... 2816

J

Jaberi-Douraki, M ... 3511
Jablonski, R ... 2623
Jack, J R ... 2751
Jackson, A ... 3438
Jackson, D ... 2235
Jackson, G R ... 3396
Jackson, K L ... 2384*
Jackson, M ... 1228, 2007, 2346, 2477
Jackson, M C ... 1199, 1284, 1313
Jackson, M P ... 2993*
Jackson, T ... 3083
Jackson-Humbles, D N ... 1755*
Jacob, B ... 3097
Jacob, R E ... 2216
Jacob Roy, R ... 2127
Jacobs, A ... 1614, 2202, 2574*
Jacobs, H ... 3291
Jacobs, H M ... 3294*
Jacobs, K ... 2163
Jacobs, M ... 1728*
Jacobs, M N ... 3001
Jacobsen, B ... 1606
Jacobsen, M ... 2685
Jacobsen, N R ... 2808*
Jacomasso, T ... 2860
Jacques-Jamin, C ... 3512
Jacquet, A ... 2372
Jaeschke, H ... 1234, 1971, 1981, 2319, 2491, 3092, 3178
Jagannathan, L ... 2322*, 3210
Jager, S ... 1113
Jaiman-Cruz, R ... 1578
Jain, A K ... 2126*
Jain, G ... 2212, 2596
Jaing, C J ... 3428
Jaligama, S ... 1439, 2245*
James, A K ... 1583*
James, L ... 2489
James, L P ... 1110
James, R A ... 2170
James-Yi, S A ... 2935*
Jan, Y-H ... 2097*, 2099
Janardhan, K ... 2131
Jang, Y ... 2880*
Janicke, B ... 3545
Jannasch, A S ... 1792

Jannuzzi, T ... 1321
Janovitz, E ... 1668, 1676, 2726, 3091
Jansen, E H ... 3015
January, C T ... 1745
Jarabek, A M ... 2713, 3366*
Jarema, K A ... 1795
Järnberg, J ... 2142
Jaruga, P ... 1390, 1404, 1417
Jaskot, R ... 1067, 2266
Jaspers, I ... 1338, 2490, 2960*
Jatana, S ... 1503
Jaunky, T ... 3030
Jaworska, J ... 2195, 2196
Jayaraman, S ... 2933
Jayasundara, N ... 1848*, 2951, 3284
Jayyosi, Z ... 1093
Jean, P A ... 2703*
Jefcoate, C R ... 3142, 3149, 3151
Jeffay, S ... 1822
Jefferson, A M ... 2346
Jefferson, W N ... 3231
Jeffery, B ... 3078, 3079
Jemth, A-S ... 1417
Jenkins, A F ... 1538
Jenkins, G ... 2772
Jenkins, J A ... 2931*
Jenkins, S ... 3340
Jenner, J ... 2101
Jennings, M ... 1303
Jennings, P ... 1713*
Jenny, M J ... 3295*
Jensen, E ... 2445
Jensen, H M ... 1124
Jensen, M L ... 3242
Jenson, A B ... 2496
Jeong, C ... 1872
Jeong, H G ... 1460*, 1469, 1470, 1472, 1473, 1486, 1488, 1489
Jeong, H M ... 1460
Jeong, K S ... 1424, 3429
Jeong, T C ... 1424, 3429
Jeppesen, G ... 3242, 3524
Jessen, B ... 1667
Jessop, F ... 1523*
Jestoi, M ... 3008
Jett, D ... 2103
Jett, D A ... 2093, 2102, 2404
Jeurink, P ... 2873
Jewo, P ... 1463
Ji, J ... 2171
Ji, K ... 2947
Ji, Y ... 3206
Ji, Z ... 1426*, 3498
Jia, F ... 2955
Jia, K ... 2292
Jia, Y ... 2906
Jia, Z ... 2022, 2329*, 2763, 2821
Jiang, H ... 1652, 1657
Jiang, W ... 2366, 3152, 3158, 3160, 3465
Jiang, Z-Y ... 1863
Jiko, H ... 1826
Jimenez, J A ... 1595*
Jimenez-Córdova, M I ... 3130
Jiménez-Garza, O A ... 3219*
Jin, C ... 1890*, 2064, 2072, 3201*, 3338
Jin, G B ... 1362
Jin, G-B ... 1365
Jin, H ... 2341, 2350, 2351, 2373, 2374, 2375, 2396
Jin, S W ... 1470, 1472, 1473*, 1486, 1489
Jin, U ... 2309*
Jin, U H ... 2056
Jin, U-H ... 2066
Jin, Y ... 2746, 2747
Jing, H ... 1810
Jing, J ... 3446
Joakim, A ... 1137*
Joglekar, R ... 3077*
Johanning, K M ... 2925
Johänning, J ... 3543
Johanson, G ... 1282, 1944, 2142*
Johari, S A ... 2940
John, A ... 1896
Johne, S ... 1106, 3036
Johnson, B C ... 1686
Johnson, B P ... 3266*, 3388
Johnson, C ... 1671
Johnson, D ... 2662*, 2998
Johnson, D E ... 2620, 2622, 2658
Johnson, E ... 1808, 1849
Johnson, G ... 1684, 2663, 2854, 2999, 3000, 3174
Johnson, J ... 1605
Johnson, J A ... 2361*
Johnson, J R ... 2461
Johnson, K ... 1120*, 1869, 2482, 3558
Johnson, N M ... 3214
Johnson, R ... 1086
Johnson, T ... 1677
Johnson, V J ... 2864*
Johnston, A S ... 1333*
Johnston, C J ... 1290, 1903
Johnston, N ... 2721
Johnston, T K ... 2933
Johnstone, A F ... 1068, 1200
Jolley, H ... 1526
Jolly, R A ... 1108
Jonaitis, T ... 2667
Jonassen, H ... 3478
Jones, A D ... 1103
Jones, B C ... 2336
Jones, C D ... 2490
Jones, D ... 1763*, 2319, 3360*
Jones, D P ... 1125, 1245, 1529, 2326, 2360, 2730, 3421, 3537*
Jones, K ... 2272
Jones, M Y ... 2799
Jones, P ... 2596
Jones, R A ... 3430
Jones, R S ... 3419
Jones, S W ... 2490
Jones, T K ... 1232
Jones, Y ... 2774
Jonigk, D ... 2710
Jonsson, M ... 3008
Jordá-Beneyto, M ... 3020
Jordan, A ... 1562*
Jordan, S ... 3042
Jordt, S E ... 1046b*, 1301
Jorge, K ... 2408*
Jortner, B S ... 1659, 2935
Jos, A ... 3020*, 3022*, 3023*
Jose, C ... 3210
Jose, C C ... 2322
Joseph, L B ... 2094*
Joseph, P ... 1921*
Joshee, L ... 3120
Joshi, A D ... 2427*
Joshi, K ... 2330*
Joshi, N ... 1951, 1954, 1965*
Joshi-Barve, S ... 1976
Joy, M S ... 2721, 3114
Ju, C ... 1332
Juan, C ... 2276
Juan, S-A ... 3068
Juan-Garcia, A ... 3067
Juarez, A L ... 1195
Juárez Hernández, U ... 1978
Judge, J L ... 1294*
Judson, R ... 1077, 1098, 1803, 2547, 2660*, 3017, 3300, 3365*, 3539
Judson, R S ... 1886, 2161, 2616
Jugloff, D ... 1454
Juhasz, K ... 1745, 1829
Julien, S ... 3440
Junaid, S ... 1164
Jung, B H ... 2872
Jung, B-H ... 2871
Jung, E-C ... 3509, 3510
Jung, G L ... 1215
Jung, R ... 1862
Jung, S H ... 1233*
Jungnickel, H ... 2541
Jurban, M ... 1137
Jüsten, P ... 2268
Jutooru, I ... 2026, 2056

K

Kaaber, K ... 3242
Kabilan, S ... 2144, 2215, 2216
Kadar, T ... 2129
Kadlubar, S ... 2746
Kado, N ... 1423*
Kado, S ... 1366
Kadonosono, T ... 3102
Kadry, A-R M ... 2984*
Kaelin, B R ... 1984
Kagan, V E ... 1291, 2803
Kagawa, T ... 3102
Kageyama, Y ... 3432
Kahle, J S ... 2490
Kahlig, K ... 2826*, 2829
Kahru, A ... 2814
Kai, K ... 1202
Kaina, B ... 1062*
Kaisar, M A ... 1621
Kaiser, J P ... 2159, 2213
Kaiser, R ... 1646, 2832
Kakade, S ... 1617
Kakehashi, A ... 1092, 2031*
Kakeyama, M ... 1190
Kakuni, M ... 3432
Kalata, E ... 1169
Kalidindi, S R ... 2473
Kalkunte, S ... 2032
Källman, T ... 2807
Kallon, S ... 2392
Kalmes, R ... 2661
Kalra, O P ... 2910
Kaluarachichi, M R ... 1107
Kalueff, A V ... 1661
Kalyan, R ... 3128
Kamendulis, L M ... 3414*
Kaminski, N ... 1364, 1736, 2686, 2687
Kaminski, N E ... 1118, 1340, 1341, 1360
Kamisnki, N E ... 1342
Kamp, H ... 2582*
Kamp, H G ... 1115, 2463*, 3054, 3057, 3058, 3536
Kan, H ... 2830*
Kan, L ... 1057, 1869
Kan, R K ... 2095
Kanda, D ... 2284*
Kanda, Y ... 1827*
Kandasamy, K ... 3075
Kandeel, F H ... 3257
Kandel Gambarte, P ... 2901
Kaneko, T ... 1476
Kanerva, J ... 1653, 1671
Kaneto, M ... 3136
Kang, G ... 2944
Kang, H-G ... 3068, 3074
Kang, J ... 3488
Kang, K-W ... 1233
Kang, M ... 2859
Kang, M J ... 1424, 3429*
Kang, M-J ... 2871, 2872
Kang, M-s ... 2272
Kanki, M ... 2001
Kannan, A ... 1735
Kannan, R ... 1504
Kanno, J ... 1838, 2613*
Kant, R ... 2115, 2116, 2121, 2126
Kanthasamy, A ... 2120, 2341, 2350, 2350, 2351, 2373, 2373, 2374, 2374, 2375, 2375, 2396, 2396
Kanthasamy, A G ... 2341, 2351
Kapeles, J ... 2136
Kapidzic, M ... 3236
Kaplan, B ... 2869*
Kaplan, B L ... 1170
Kaplan, B L F ... 3354*
Kaplan, D ... 1137
Karakaya, M A ... 2813*
Karakitsios, S ... 1698, 2472
Karchner, S I ... 2424
Kare, P ... 2910
Karelia, D N ... 1328*
Karimi, S ... 2796*
Kariyazono, Y ... 1868
Kark, A ... 1980*
Karkare, S ... 2356
Karki, K B ... 2056*
Karki, P ... 2361, 2362
Karlsson, H L ... 2788, 2807
Karmaus, A L ... 2637*
Karmous, O ... 2483
Kartashov, A V ... 2322
Karvonen, A ... 1851
Kasai, H ... 3197
Kasemets, K ... 2814*
Kashon, M ... 2773, 2782, 2830
Kashon, M L ... 1199, 1558, 3485
Kasting, G ... 2532*
Kasturi, P ... 1373*
Katavolos, P ... 1666
Katner, A ... 2986*
Kato, H ... 3482
Kato, R ... 2776
Kato, Y ... 3136*
Katoh, H ... 1826
Kauffman, M E ... 2022
Kaur, G ... 1908*, 2008
Kaur, J ... 2254
Kaur, K ... 1846*, 3248
Kaur, M ... 2127
Kaushik, S ... 1658
Kauss, A ... 1743
Kavanagh, T ... 2323, 2324
Kavanagh, T J ... 1499, 2181
Kawabe, M ... 2033
Kawada, T ... 1348
Kawai, K ... 3093, 3197*
Kawakami, T ... 2776
Kawamoto, T ... 2965
Kawamura, K ... 1604
Kawamura, T ... 2156
Kawasaki, T ... 1476
Kay, V ... 3501
Ke, S ... 2304, 2437
Ke, Y ... 3168
Kearsing, L ... 1894
Keating, A ... 3226
Keating, J H ... 2441*
Keebaugh, A ... 2264, 2265
Keech, S ... 1624
Keefe, D ... 1370*, 2958*
Keel, A ... 3531
Keely, A ... 2415
Keene, A ... 2544
Kegels, A ... 2723
Keil, K ... 1191, 1193
Keil, K P ... 1192*
Kelada, S ... 1311*
Keller, D ... 2475
Keller, J ... 2537, 2541
Kelley, K M ... 2457
Kelley, S ... 2999*
Kelly, A D ... 1554
Kelly, E ... 3119
Kelly, E J ... 1105, 1989, 3110, 3207
Kelly, K A ... 1211, 1212, 1214*, 2918
Kelm, J M ... 1669, 2000, 2173, 3460
Kelman, B ... 2277, 2671, 2696
Keltner, Z ... 2771
Keltner, Z M ... 2789
Keminer, O ... 1742
Kemmerling, B ... 1627
Kempa, S ... 2338
Kenedi, C ... 2828
Kennedy, G ... 2076
Kennedy, R T ... 2523*
Kennedy, W ... 1638
Kenneke, J F ... 3413
Kennell, A ... 2539
Kenney, C ... 3278
Kennon-McGill, S ... 1234
Kensler, T ... 1760*
Kenyon, E M ... 2231*
Kerger, B D ... 2979
Kermanizadeh, A ... 2808
Kern, C ... 2457
Kern, P ... 2195
Kerns, W ... 3085
Kerper, L E ... 1692
Keski-Nisula, L ... 1851
Kessler, V ... 2171
Kettenhofen, R ... 1742
Keuzenkamp, E J ... 1511
Key, P ... 2221
Khalaf, A E A ... 2914
Khan, I A ... 1487, 3194, 3282
Khan, M F ... 2870, 3191
Khan, N ... 2942
Khan, S ... 2321
Khaniya, B ... 2493
Khanna, A ... 2212
Khanna, M ... 2333
Khare, S ... 1514, 3026
Khedr, M E ... 2333
Khoo, L H ... 2935
Kiany, A ... 1164
Kidd, K ... 2412
Kidd, K A ... 2952
Kido, T ... 3133*

The numerals following the author names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the presenter.

Kiefer, A ... 2496
Kienesberger, P C ... 2412
Kieper, J ... 1625
Kii, H ... 1824
Kijanska, M ... 1669
Kijima, A ... 2050
Kikkawa, R ... 2605
Kikuchi, T ... 3451
Kilarkaje, N ... 3254*
Kilic, M ... 2748
Killic, O ... 1148
Kim, C B ... 1608
Kim, C H ... 1798
Kim, D ... 1738, 2588, 2729, 2889
Kim, D G ... 1474
Kim, D J ... 2065*
Kim, D-S ... 1226*, 2119, 2120
Kim, H ... 1137, 2778
Kim, H D ... 2094
Kim, H G ... 1460, 1470
Kim, H R ... 1287, 1306*
Kim, H S ... 2027*
Kim, H-J ... 1464*
Kim, J ... 1089, 1942, 2476, 2778, 3460
Kim, J J ... 1306, 1420*, 2770*, 2777*
Kim, J W ... 1191
Kim, J-S ... 3169
Kim, K ... 1738*, 2066, 2792, 2947*
Kim, K S ... 2027, 2749
Kim, M ... 2065, 2626
Kim, M T ... 1400*
Kim, S ... 2729
Kim, S J ... 1470, 1473, 1489*
Kim, S-H ... 1468*, 1517
Kim, T ... 3526*
Kim, W-K ... 2948*
Kim, Y ... 2588, 2889
Kim, Y H ... 1486, 1489, 2266*
Kim, Y-H ... 1512, 3217*
Kimber, I ... 1939, 1947, 1948*
Kimber, M ... 2350
Kimmitt, R ... 2727
Kimura, E ... 1190*
Kimura, M ... 2028
Kimura, Y ... 2806
Kincaid, J ... 1143
Kindrat, I ... 1059, 2037*
Kindzierski, W ... 2997
King, B ... 1686
King, B L ... 1086
King, C ... 1065, 1070, 1448, 2262, 2266, 2838
King, R ... 1790
Kinniburgh, D W ... 1435
Kinoshita, K ... 3456
Kinoshita, S ... 2920
Kinter, L ... 1627
Kipen, H ... 1297*
Kirby, I ... 2937
Kirby, M P ... 1103
Kirk, S A ... 1654*
Kirkley, K ... 2359*
Kirman, C R ... 2237*
Kirsch, T ... 2197
Kisin, E R ... 1291, 2803*
Kiss, L ... 2096, 2127
Kissick, H T ... 3138
Kissling, G ... 1353, 2131, 3089, 3271
Kissling, G E ... 3177, 3389
Kistinger, B ... 1381, 2895*
Kitajima, S ... 2613
Kitano, H ... 1113
Kitazawa, M ... 1586*
Kitazawa, T ... 3289
Kiyono, T ... 3148
Kiyota, Y ... 1824
Kizaka-Kondoh, S ... 3102
Klaassen, C D ... 1934, 1969, 3144
Klapacz, J ... 1881*
Klaren, W D ... 3416*, 3420
Klaric, M ... 1616*, 3512
Klaunig, J ... 2478, 3370*
Klaunig, J E ... 1058, 1456, 2041, 2042, 3184
Klausner, M ... 3066, 3069, 3070, 3516
Kleemann, R ... 1983
Kleensang, A ... 1084, 3408
Kleider, C ... 1116, 1449, 3391
Klein, B ... 2483
Klein, C ... 1644
Klein, C B ... 1165
Klein, D M ... 1985
Klein, R L ... 2384
Klein, U ... 1638
Kleindienst, T E ... 1448
Kleinjans, J ... 1007*
Kleinjans, J C ... 2612
Kleinman, M ... 2264
Kleinman, M T ... 2265
Kleinoder, T ... 2709
Kleinstreuer, N ... 1098, 2202, 2224, 2651*
Kleinstreuer, N C ... 2533*
Klemm, L C ... 1924*
Klinefelter, K ... 1195*
Klingelhutz, A J ... 3420
Klocke, C ... 1166
Klocke, C R ... 1167*, 1188
Klocke, Z ... 2797
Klug-LaForce, M ... 1398
Kluz, T ... 1562, 2064, 2072, 2075, 2322, 3218, 3338
Knap, T ... 3433
Knapp, A ... 3434
Knapton, A ... 3473
Knebel, J ... 1510, 1941, 2016, 3548
Knecht, A L ... 1849*, 3287
Knecht, D A ... 3551
Kneuer, C ... 1498
Knight, D ... 2525*, 2573b*
Knight, R ... 1270, 2685*
Knoflach, F ... 1645
Knolle, P ... 2500*
Knoll-Gellida, A ... 1806
Knox, B ... 2746, 2747
Knox, T ... 1658
Knuckles, T L ... 2962
Knudsen, G A ... 2045, 3406*
Knudsen, T ... 2547, 2640*, 3300
Knudsen, T B ... 1104
Ko, C-I ... 1737*, 1823
Koba, H ... 3456
Kobayashi, A ... 3182
Kobayashi, E ... 2001
Kobayashi, M ... 1348
Kobets, T ... 1820, 1821*
Kocarek, T ... 3457
Kochmanski, J ... 3203*
Koci, J ... 3078, 3079
Kodali, V ... 1289, 1509, 3488, 3496
Kodali, V K ... 1526, 3485*
Kodama, Y ... 1960
Kodavanti, P R S ... 1200*
Kodavanti, U ... 1066, 1067, 3195
Kodavanti, U P ... 1068, 1069, 1281, 2135*
Koehler, M W ... 1426
Koenig, C ... 1395*
Koenig, J A ... 2095*
Koetzner, L ... 3011
Koganezawa, N ... 1172
Kogel, U ... 3401*
Koh, J ... 2412
Koide, F ... 2108
Koivisto, P ... 3008
Kojima, H ... 1604*, 2203, 2471
Kojo, A ... 2988
Kokkonen, U-M ... 3008
Kokkotou, E ... 2912
Kolasa, E ... 2046, 2057*
Kolin, C ... 1646
Kolle, S N ... 2192*, 2200, 2201
Kolli, R ... 3135*
Köllmann, C ... 1116
Kolossov, E ... 2857
Komaki, T ... 1931
Komoriya, K ... 2776
Kondakala, S ... 2899*
Kondo, C ... 3136
Kondo, K ... 3182*
Kondou, C ... 3249
Kondraganti, S ... 1492*
Kondratyev, A ... 1247
Kondru, N ... 2374
Kong, B ... 1910, 1953
Kong, J ... 2833
Kong, L ... 2367, 2600
Kong, M J ... 3429
Kong, Q ... 2061*
Konneker, T ... 2546
Kono, D H ... 2874
Kontaridis, M I ... 2855
Kooter, I M ... 2815*
Kopacz, A ... 2390
Kopec, A K ... 1951, 1954*, 1965
Kopia, G A ... 2442
Koponen, I K ... 2248
Korchevskiy, A ... 1286*
Korgaonkar, C ... 3477
Koriyama, C ... 1567
Kornberg, T ... 2783
Korpela, S ... 1439
Korpela, S P ... 2844
Korrapati, M C ... 1982
Korte, S ... 1618, 1633, 1639*
Kosaka, S ... 3277
Koschmann, J ... 2710
Koschnitzky, J E ... 1177
Koshita, M ... 1604
Koshlukova, S ... 2884, 2900
Kostal, J ... 2323, 2324
Kosyk, O ... 2729, 3434
Kothiya, P ... 2640
Kotora, K ... 2473
Kottmann, R M ... 1294
Kouzuki, H ... 2204
Kovacevic, R ... 2945
Kovalchuk, N ... 3147, 3458*
Kovalova, N ... 1364*
Kovari, A ... 2988
Kovarich, S ... 2656
Kovochich, M ... 1310, 2695
Kozal, J S ... 1848, 2951, 3284*
Koza-Taylor, P ... 1073*
Kozhich, A ... 3440
Kracko, D ... 2086, 2258, 2742, 3395
Kraeling, M E ... 2789
Kraft, A ... 1198
Kraft, A D ... 2707*
Krähenbühl, S ... 1972
Kramer, S ... 3081
Krantz, Q ... 1065, 1070
Krantz, Q T ... 1448, 2266
Krantz, T ... 2838
Krapfl, H ... 1452
Kraus, W L ... 1359
Krause, M ... 2671
Kreider, M ... 2976
Kreider, M L ... 1864*
Kreilein, C ... 1627
Kreiling, R ... 2544
Kreiss, K ... 1278
Kreitinger, J M ... 1361*, 1367
Krennrich, G ... 1115, 2463, 3536
Kreuger, S ... 3161
Krevitz, J A ... 2024
Krieger, L K ... 3411, 3412*
Kringstad, J ... 2446
Krishna, G ... 1637*
Krishnamurthy, P ... 3341
Krishnan, K ... 1096
Kristofco, L ... 2323, 2324
Kristovich, R ... 3103
Kroeker, R ... 3278
Kroll, K J ... 2939
Krolski, M D ... 2897
Kromme, M ... 1195
Krone, P H ... 1583
Kroner, O ... 3410
Krueger, W ... 2013
Krug, J ... 1065, 1070, 1448, 2838
Kruger, O ... 2474
Kruhlak, N ... 1400
Kruijssen, L ... 1334
Kruse-Jarres, R ... 2968
Kruszewski, M ... 1299
Kruzich, P ... 1627
Krygsman, A ... 1893
Krzyzewski, S ... 3061
Kubaszky, R ... 1208, 3105*
Kubátová, A ... 1450
Kuberry, I ... 3024
Kubo, K-i ... 1190
Kubota, A ... 1799
Kuchimaru, T ... 3102
Kuehl, P ... 3395
Kuehl, P J ... 2258*
Kuehn, D ... 3036
Kuga, K ... 3522*
Kuhlbusch, T A ... 1518
Kuhlman, C ... 2146*
Kuhn, D D ... 2935
Kuhn, E A ... 3428
Kukuruzinska, M ... 1732
Kulick, C V ... 1247
Kulkarni, R ... 1395, 1398
Kulkarni, S R ... 1966*
Kulling, S ... 1116, 1449
Kullman, S W ... 1175, 1882, 2892, 3288
Kulp, K ... 1153, 2390
Kumagai, K ... 1063, 1100
Kumagai, Y ... 1563, 1582, 2295, 2298, 3451
Kumagai-Takei, N ... 1354, 1355, 1356, 1357, 1358*, 2784
Kumanchik, L ... 1228, 2007, 2477
Kumar, D ... 2115, 2116, 2121*, 2126
Kumar, G ... 2450*, 2453, 2762, 2764
Kumar, K K ... 2364
Kumar, M ... 1974, 2493*
Kumar, R ... 2212, 3061, 3156*
Kumar Barupal, D ... 2049
Kumarathasan, P ... 1268
Kummer, E ... 2861
Kumpf, S W ... 1801
Kumsher, D M ... 1541
Kundu, R ... 1916
Kung, H M ... 2648*
Kung, M H ... 3441
Kuno, H ... 3182
Kunz, A ... 1222
Kuo, L-J ... 1697, 3404
Kuper, F ... 2815
Kuprat, A P ... 2144, 2215*, 2216
Kupsco, A ... 2955, 3290*
Kurata, K ... 1838
Kurata, M ... 2603
Kurhanewicz, N ... 2260*, 2838
Kuroda, Y ... 2732*
Kuronuma, M ... 3093
Kurtz, S ... 1627
Kusaka, M ... 1355
Kuseryk, L ... 1641
Kushida, M ... 1092
Kusunoki, Y ... 1838
Kutepova, V ... 1392
Kutzner, B C ... 3533
Kuwata, C ... 1202
Kuwata, K ... 1960*
Kwakye, G ... 1595, 2342, 2389, 2391*, 2392
Kwiatkowski, C F ... 1859
Kwok, E ... 2900
Kwon, O-S ... 2871, 2872
Kyrlidis, A ... 3496
Kyrtopoulos, S A ... 2612

L

La, C ... 1423
La, H ... 1666
La Du, J K ... 1805
La Merrill, M ... 1174, 2545
La Merrill, M A ... 1850
Labrèche, F ... 2693
Lac, D ... 1619, 3514*
Lacey, A ... 2418*
Lacey, C J ... 2113
Lacher, S E ... 2307*
Lacy, S ... 1911
Lader, E ... 3472
Lafond, J-F ... 1246
Laforce, M K ... 2053
LaFranco-Scheuch, L ... 1677
Lafuente, D ... 2795
Låg, M ... 1450
Lagadic-Gossmann, D ... 1450
Lai, K ... 2025
Lai, Y ... 1425*
Laine, J ... 1259
Laine, J E ... 2977*
Laing, S ... 1611
Laird, B D ... 2285*
Lakin, J ... 1601
LaKind, J ... 1038*
Lalayeva, N ... 3090*
Lalko, J ... 3520

Lallemand, M-S ... 3237
Lalu, M ... 2632
Lam, H ... 3556
Lam, K S ... 1619
Lamantia, C E ... 1180
Lamar, P C ... 3122
Lamas, N ... 2407
Lamb, J C ... 1179
Lambrechts, N ... 1142*
Lamm, M ... 2209
Lamm, S ... 2149*, 2983
Lammens, L ... 2723
Lamoureux, C ... 3162
Lamparelli, D ... 2741
Lampe, B ... 3007*
Lampe, B J ... 2154
Lampe, J N ... 1952
Lamure, S ... 2483
Lamyaithong, A B ... 3126
Lan, H ... 3531
Lan, Q ... 2923
Lanceta, J ... 1143
Landenberger, B ... 2226
Landis, J ... 3272
Landis, K ... 1625
Landis, M ... 2266
Landolph, J R ... 2081*
Landry, G M ... 1271*
Landry, T ... 3069, 3070*
Landsiedel, R ... 2043, 2192, 2200, 2201, 2223, 2537, 2541, 2544*, 3052
Landuyt, B ... 3460
Laney, J ... 2100
Lang, A L ... 1984*
Lange, S ... 2994
Lange, S S ... 2992*
Langenberg, K ... 1744, 1830
Langer, J ... 1228, 2007, 2477
Langley, M ... 2120
Langley, M R ... 2374*
Lanigan, C ... 1671, 1672
Lankoff, A ... 1299
Lannay, Y ... 1636
Lanning, L ... 3087*
Lansdell, T ... 2008
Lantz, C ... 2602
Lantz, R C ... 2570*
Lantz-McPeak, S M ... 2347
Lanz, H ... 3113
Lao, W ... 2955
Lao, Y ... 1605
LaPres, J J ... 2686*, 2687
Larard, S ... 3031
Larbaoui, M ... 2618
Large, C ... 2961*
LaRocca, J ... 1057, 1891*
Larsen, L ... 1997
Larsen, M C ... 3149, 3151
Lasher, M ... 2882
Lasher, M A ... 2473
Laskin, D ... 1953, 2118, 2560*
Laskin, D L 1910, 1915, 2094, 2097, 2099, 2113, 3348, 3350
Laskin, J ... 2118
Laskin, J D 1910, 1915, 2094, 2097, 2099, 2113, 3233
Latvala, S I ... 2788*
Lau, C ... 3439*
Lau, S S ... 1244, 1599, 2082, 2380*, 2599
Lau-Cam, C A ... 2330
Laue, H ... 2925
Lauer, F T ... 1350, 1352
Lauerre, V ... 2077
Laukova, M ... 1173, 1220*
Laumbach, R ... 1297
Lauster, R ... 2187
Lauterbach, J H ... 2460*
Lauterstein, D ... 1167, 1847
Lauterstein, D E ... 1165*
Laux, P ... 2541, 3525
Lavado, A ... 1228, 2477*
Lavallée, S ... 1415
Lavell, E ... 1148
Lavine, J E ... 3179
Lavoie, J ... 2693
Lavoie, M ... 3390*
Lavrich, K ... 2210
Lavrich, K S ... 2320*
Lawana, V ... 2396
Lawana, V J ... 2373*
Lawhon, S ... 2437
Lawless, M S ... 1099
Lawrence, B P ... 1359, 1365, 1368
Lawrence, D A ... 1894*, 3551
Lawrence, P ... 1362
Laws, S C ... 1887, 1888, 1889, 2933
Lawson, M ... 2006
Lawton, M ... 1073
Lawton, R I ... 3397
Layko, D ... 2036
Le, G T ... 2082
Le, H ... 1619
Le, N N ... 3080
Le, V ... 1336
Le, X C ... 1478
Le, Y ... 2302, 3180*
Le Bihanic, F ... 1806
le Ferrec, E ... 1450
Le Godec, T ... 2737
Le Net, J-L ... 3493
Leach, C A ... 2091, 2109
Leal, R B ... 2357
Leane, L ... 3391
Leavitt, P ... 1399
LeBaron, M ... 1120, 1869
LeBaron, M J ... 1057*, 1426, 1891
Leblanc, A ... 2430, 3427
Leblanc, V ... 1612, 1613, 1614*
Lebofsky, M ... 1971
Lebrun, A ... 2249
Lebrun, S ... 3400
LeCluyse, E ... 2005, 2018, 3175
LeCluyse, E L ... 1996, 2014, 2432, 3181, 3464
Ledbetter, A ... 1068, 1429, 2260
Ledbetter, A D ... 1069, 2135, 2263
Lednicky, J ... 2787
Lednicky, J A ... 2811
Ledvina, A ... 3532
Lee, B-H ... 1233, 1474
Lee, B-J ... 2859*
Lee, C-W ... 2778
Lee, D Y ... 1812
Lee, E ... 2361, 2362, 2367*
Lee, E-H ... 2724*
Lee, G H ... 1488*
Lee, G I ... 2245
Lee, H ... 2065, 2778*
Lee, H S ... 1460
Lee, J ... 1588*, 2307
Lee, J S ... 2572*, 2700
Lee, J W ... 1232, 2345, 3501
Lee, J-H ... 2899
Lee, J-S ... 2158
Lee, J-Y ... 1528*, 1532, 1534, 1566
Lee, K ... 2778, 2859
Lee, K H ... 2365
Lee, K Y ... 1460
Lee, M ... 3486, 3547
Lee, S ... 1354, 1355*, 1356, 1357, 1358, 1468, 1517*, 1653, 1671, 2019, 2729, 2784, 3169
Lee, S H ... 2872
Lee, S-M ... 1546
Lee, W-K ... 1546
Lee, Y C ... 1470
Lee, Y S ... 1270
Leet, J ... 1797
Lefebvre, F ... 3196
Lefebvre, W ... 1854
Lefevre, M ... 2268
Legare, M E ... 1917
Legé, P ... 2835
Legouffe, R ... 1293
Le-Grand, B ... 2430
Legters, G ... 2007, 2851
Lehman, B ... 3091
Lehman, J G ... 1601
Lehman-McKeeman, L ... 1668, 2498*, 3091
Lehman-McKeeman, L D ... 2726
Lehmann, D M ... 2202
Lehmann, L ... 1116, 1449
Lehmler, H-J ... 1193, 3425
Lehoux, S ... 3344
Leigh, H ... 3513
Leigh, N J ... 3397*
Leigh, P ... 3346
Lein, P ... 1193, 1229, 2105, 2409, 2410, 2506*, 3315*
Lein, P J ... 1191, 1192, 1197, 1884, 1892, 2395, 2416
Leist, M ... 1171, 2338, 2381, 2583*, 3313*
Lemaire, B ... 1799
Lemaire, M ... 3344
Lemarié, C A ... 3344
Lemay, J ... 2673
Leme, D M ... 2860*
Lemkuhl, D ... 2356
Lemus, R ... 2993
Lena, C ... 2743
Lenburg, M ... 3396
Leonard, J ... 2221, 2222*, 2238
Leonard, S ... 1314, 2780
Leonard, S S ... 1558, 3496
Leong, J ... 3239
Leonhardt, A ... 3499
Leon-Rodriguez, R ... 1219
Lepist, E I ... 2829
Lerner, C A ... 3398*
Lerory, J ... 2272
Leroux, F ... 1415
Leroy, P ... 3029, 3036, 3402, 3552
Less, R ... 2004*
Lesueur, F ... 3259
Letzig, L ... 2489
Leung, C-L ... 1861
Leung, M C ... 1104*
Leventhal, M ... 1230
Levesque, P ... 1674
Levin, E D ... 1163, 1164, 1175, 1848, 3284
Levin, I ... 1505
Levoe, S N ... 2395
Levy, G ... 1980
Lewandowski, M ... 1448
Lewandowski, R ... 2130
Lewandowski, R J ... 2303*
Lewandowski, T A ... 2670, 2989*
Lewin, M D ... 3419
Lewine, J ... 2100
Lewin-Koh, S ... 3481
Lewin-Koh, S-C ... 1638
Lewis, B W ... 1935
Lewis, C ... 2900
Lewis, C A ... 2069*
Lewis, C M ... 2715*
Lewis, E ... 1336
Lewis, J ... 1132, 1559, 1560, 1635, 2981*
Lewis, J A ... 1270*, 1541
Lewis, M M ... 2367
Lewis, S ... 3553
Lewis, S C ... 2727
Li, A A ... 2411
Li, A P ... 1995, 3145, 3146*
Li, C ... 1597, 2826
Li, C Y ... 2300, 3270*
Li, D ... 1329, 2304, 2622*
Li, F ... 1961, 3059, 3554*
Li, G J ... 1810*
Li, H ... 1084, 1572, 2407, 2548*, 3179*, 3201, 3345
Li, J ... 1198, 1319*, 1360*, 1652, 1656, 2149, 2983, 3051, 3206*
Li, J Z ... 2022
Li, K ... 1671
Li, K L ... 2666*
Li, L ... 1656, 3147
Li, M ... 1109*, 1196, 1483, 1840, 3516
Li, P ... 2967
Li, Q ... 1348*, 1914*
Li, S ... 1519, 1529, 2319, 2730*, 3121*, 3421*
Li, W-K ... 1572, 3171
Li, X ... 1058, 1193, 1661*, 2041*, 2042, 2080*, 2134
Li, X-F ... 1319, 1478
Li, Y ... 1109, 1831, 2731*, 2758, 3075
Li, Y R ... 2022*, 2329, 2821
Li, Y-J ... 1183
Li, Y-S ... 3197
Li, Z ... 1213, 1657, 3474*
Liang, L E ... 3348
Liang, L-P ... 1209, 2406*
Liang, S ... 3251*, 3252, 3539
Liang, Y W ... 3154
Liaw, J-J ... 3256
Lichter, J ... 3517*
Lichti-Kaiser, K ... 2018
Lichtler, R ... 1272, 1550*
Lichtveld, M ... 1054*, 2257
Licollari, A ... 3172*
Lidberg, K ... 1989*
Liedtke, W B ... 1920
Lightbody, E D ... 3094*
Lilius, E-M ... 3008
Lim, A ... 3025
Lim, J ... 3246
Lim, J-M ... 1546
Lima, B T ... 2855
Limbeck, E ... 2085*
Limberis, J ... 2826
Limon-Pacheco, J H ... 1219
Lin, G ... 3185
Lin, G X ... 2346
Lin, L ... 1269
Lin, P ... 1899
Lin, P-Y ... 1412, 2071*
Lin, Q ... 2349*
Lin, S ... 1232, 2345
Lin, T-M ... 2055
Lin, Y ... 1545
Lin, Y-S ... 2984
Lin, Z ... 1329, 1504*, 2843, 3079
Lin, Z Y-C ... 3148
Linardic, C ... 2074
Linares, V ... 2315
Lindberg, C D ... 1848, 2951*, 3284
Lindberg, T ... 2205
Lindon, J C ... 1107
Lindstedt, M ... 2205*
Lindstrom, A ... 3439
Ling, W ... 1190
Lingappan, K ... 3143*, 3154
Linn, S ... 2356
Linnenbrink, M ... 2606*
Liong, M ... 2695
Liou, B ... 2356
Lipinski, R J ... 3266
Lippi, L L ... 1853
Lipscomb, J ... 2712
Lisboa, C ... 3296
Liska, D ... 2457
Liska, R ... 2451
Lison, D ... 2249
Litoff, E J ... 3156
Litster, S ... 2618
Littke, M ... 1526
Liu, C ... 2843
Liu, F ... 1240*, 1241, 1242
Liu, G ... 2826
Liu, H ... 2906
Liu, J ... 1114, 1237, 1475*, 1479, 1572*, 1678, 1809, 2106, 2161, 2261*, 2609*, 3059, 3082, 3084, 3088, 3171*, 3188, 3201, 3513*
Liu, J-R ... 1183*
Liu, K ... 2326
Liu, K H ... 2319*
Liu, K J ... 1275, 1352
Liu, L ... 1638, 1901*, 1950, 1961
Liu, M ... 1135
Liu, Q ... 1863, 2883*
Liu, R ... 1083*, 1619
Liu, S ... 1840
Liu, T ... 1872
Liu, X ... 2132*
Liu, Y ... 1456*, 1552*, 2064*, 2072, 2750, 3049*, 3513
Liu, Z ... 1071, 1251, 2610*, 2750, 3166, 3183, 3187, 3534
Livi, C B ... 1084*
Livingston, M K ... 3388*
Lizarraga, L E ... 2160*, 2699
Lizarraga, L L ... 1244
Llana-Ruiz-Cabello, M ... 3022, 3023
Llewellyn, C ... 1786*
Lo Piparo, E ... 2431
Lo Re, S ... 2249
Loader, J ... 2125
Lobach, A R ... 2268*
Lobbes, R ... 1442
Locker, A R ... 1211, 1212, 1214, 2918*
Lockhart, C M ... 3207*
Lødrup Carlsen, K C ... 3258

Loeb, J ... 2787
Loeb, J C ... 2811
Loehr, C ... 3161, 3246
Loehr, C V ... 2186
Loft, S ... 2248
Logan, A D ... 2314
Loghin, F ... 2276
Løgsted, J ... 3524*
Loguinov, A ... 2655, 3157
Lohstroh, P ... 2900
Loisel-Joubert, S ... 2625
London, B K ... 1623
Lone, T ... 1337
Long, C ... 2866
Long, C J ... 1228, 2007, 2477, 2851
Long, C M ... 2862*
Longnecker, M P ... 2975
Lonneman, W A ... 1448
Loo, L-H ... 3075
Loomis, D ... 1261, 1262, 2049
Looser, R ... 3536
Loots, G G ... 3428
Loots, I ... 1142
López-Amador, N ... 2325
Lopez-Carrillo, L ... 1260
Lopez-Gomez, M F ... 2956*
Lopez-Gonzalez, M L ... 1141
Lorencini, M ... 2474
Lorenz, A K ... 2187
Lorenzi-Filho, G ... 1309
Loretz, C ... 3145, 3146
Lorge, E ... 1060
Lorget, F ... 1611
Loriot, S ... 2845
Loseva, O ... 1417
Lossin, C ... 2105
Lotersztajn, S ... 3427
Loth, M K ... 2310*
Lotter, J T ... 1689
Loughery, J R ... 2952*
Louis, E ... 1020*
Louisse, J ... 2548
Loukotkova, L ... 2219
Lourens, N ... 2454
Love, R ... 3095*, 3099
Love-Homan, L ... 1664
Lovejoy, C ... 2108
Loveless, S ... 1124
Low, L K ... 3441*
Lowe, F ... 3030*
Lowenthal, M S ... 1417
Lowes, D ... 2068
Lowit, A ... 2202, 2689, 2690
Lowry, D ... 2773, 2782
Lowry, G ... 1702
Lowry, J ... 2096, 2127
Lozano, R ... 3086
Lozoya, O ... 3065
Lu, E ... 2334, 2335
Lu, J ... 2221, 2238*, 3341
Lu, K ... 3201
Lu, L ... 2336
Lu, Q ... 2449
Lu, S ... 1232*, 1667*, 2345
Lu, Y ... 3185*
Lu, Y-F ... 1678*, 3171
Lu, Z ... 2244, 2251*
Luc, R H ... 1798
Lucchini, R ... 2736
Lucchini, R G ... 2508b*
Lucero, J ... 1304, 3137
Luch, A ... 1518, 2541, 3525
Luderer, U ... 3246
Ludewig, G ... 1109, 1421
Ludwig, C ... 1181
Ludwig, F ... 1633, 1639
Luechtefeld, T ... 1084
Luerman, G ... 1225, 1742, 2857*
Luettich, K ... 3029, 3552
Lui, K ... 3495
Luis-Gonzalez, G ... 2278
Lukas, A ... 3461
Luke, A ... 1198
Lukomska, E ... 2862, 2866
Lumen, A ... 2219
Luna, L ... 1869, 2208, 2226*
Luna-Serrano, R V ... 2256*
Lund, A ... 1304, 2791, 3137*
Lundby, Z ... 1839, 2550, 3192
Lundqvist, J ... 2597, 2737*
Lunt, S Y ... 1100, 1103
Luo, B ... 1064
Luo, C ... 2489
Luo, G ... 2602
Luo, H ... 1079, 1865, 1870
Luo, J ... 2374
Luo, M ... 1879, 3125*
Luo, P ... 3125
Luo, S ... 2545
Luster, M I ... 2864
Luther, E ... 3545
Luttrell-Williams, E ... 3112*, 3116
Lutty, G A ... 1031*
Luu, K ... 3534
Luyendyk, J P ... 1951*, 1954, 1965
Luz, A ... 1403*
Lv, S ... 2963
Lv, W ... 2479
Lv, X ... 1656
Ly, K ... 2286, 2667
Ly, V ... 3479
Lydic, T A ... 1118
Lyman, M ... 1602*
Lynch, A ... 1862*
Lynch, B S ... 1149*
Lynch, H N ... 1692*
Lyn-Cook, L ... 2768
Lyn-Cook, L E ... 2753*
Lynes, M A ... 1936, 3551*
Lyon, D ... 2544
Lyons, M ... 1382*

M

M K, N ... 2596
Ma, H ... 1519*
Ma, J ... 1136, 1496, 1672
Ma, J Y ... 1653, 1671
Ma, L ... 1810
Ma, P ... 2652, 3394
Ma, R ... 2354, 2355, 3157
Mabasa, L ... 3016
Mabondzo, A ... 2791
Mabson, M ... 2717*
Macdonald, J M ... 2014, 3464
Machado, A R ... 1416
Machado, C D S ... 1416
Machin, A ... 1790
Mack, C M ... 1149
Mack, M ... 1399
MacKay, C ... 2646
Mackay, W J ... 3024
MacKenzie, D ... 1324, 1350, 1559*, 1560
Mackenzie, P I ... 3456
Mackey, A ... 2006
MacLachlan, T ... 1012*
MacLeod, F ... 2470
MacMillan, D K ... 3436
Macmillan, D S ... 1399, 2629*
Madala, H R ... 2032*
Madden, J ... 2476
Madden, J C ... 1097*
Madden, M ... 1279, 2823
Madeen, E ... 3161, 3246*
Madejczyk, M S ... 1270, 1540, 1541*
Maderdrut, J L ... 2846, 2850
Madhloum, N ... 1854
Madl, A K ... 1310, 2695*, 2978
Madnick, S ... 1084, 2174
Madraymootoo, W ... 1391
Maeda, M ... 1354, 1356
Maeda, N ... 1593
Maejima, T ... 1202*
Maël, G ... 2805
Maertens, A ... 1084, 2632, 3408
Magdziarz, T ... 2657
Mager, E ... 2934
Maghezzi, M S ... 3243
Magistrelli, M ... 2740
Magnin-Bissel, G ... 1659
Magnusson, P A ... 1299*
Magpantay, R ... 1929*
Maguire, M ... 1213, 1946, 3149
Mahalingaiah, P K S ... 2494
Mahama, B ... 1226, 2119, 2120
Mahapatra, C ... 2858
Mahapatra, D ... 1175, 1882*
Mahle, D A ... 1201*, 2734, 2887
Mahler, G ... 1516, 2781, 3111
Mahmoud, M ... 3006
Ma-Hock, L ... 2537*, 2541
Mahoney, M ... 1230
Maibach, H ... 2474, 3509, 3510
Maier, A ... 2694
Maier, M ... 2544
Maillet, E L ... 1661
Mainman, R B ... 2367
Mainquist, K ... 2194
Maisanaba, S ... 3020
Maitre, A ... 1146
Maity, S ... 3143, 3465
Maiuri, A ... 2008*, 2502
Majeed, S ... 3036
Majewska, A K ... 1180
Majumder, A ... 3076
Mak, A ... 1918*
Maki, C ... 2283
Maki, C R ... 3012*
Makia, N ... 1834*, 3339
Makin, A ... 3242*, 3524
Makley, M K ... 2887
Makori, N ... 1930
Makwana, O ... 1325*
Malarkey, D E ... 2714
Malavarca, R ... 1155
Malaviya, R ... 2113*, 2118
Malczewska-Toth, B ... 1452
Maldonado-Rojas, W ... 1088*
Malek, D ... 2446, 2448
Malfatti, M A ... 3428*
Malghan, S ... 2719
Malik, A ... 2875
Malik, M A ... 3222
Malinauskas, R A ... 2449
Maliver, P ... 1644
Malley, L A ... 3449
Mallick Bandi, P ... 3452*
Mally, A ... 2085, 3205
Maloy, E ... 3535
Manautou, J ... 1221
Manautou, J E ... 1235, 1295, 2013*, 2316, 3431*
Mancari, F ... 2741
Mandrioli, D ... 2578*
Mañes, J ... 2276
Mangelsdorf, I ... 2684
Mangipudy, R ... 3477, 3478, 3479
Mangum, L H ... 2725
Manhaes, A C ... 1243
Manikanthan, B ... 1336
Manjanatha, M ... 2769
Manjanatha, M G ... 1396
Manka, D ... 2006
Manley, M W ... 1971
Mann, K K ... 1554, 1555, 3344
Mann, S ... 1796*
Mannerström, M ... 3062
Manning, R ... 3095
Mans, D ... 1447
Mansell, P ... 3096*
Mansfield, K ... 1749*
Mansfield, L S ... 2875
Manson, L ... 1930
Mansour, A M ... 3399
Mansouri, K ... 2627
Mantecca, P ... 2786, 2814
Mantell, L L ... 1909, 1912, 1916
Manwaring, J D ... 2197, 3512*
Manzano-Leon, N ... 2253
Mao, C ... 3151
Mara, K ... 2402
Marc, P ... 2605*, 2618, 2641
Marceau, G ... 1415*, 1418
Marchan, R ... 3460
Marchitti, S A ... 3413*
Marchlewicz, E H ... 1858*, 3204
Marchlewicz, L ... 3203
Marcinek, D J ... 2936
Marco, S M ... 2400*, 3348, 3350
Marcus, C ... 3142
Marcus, C B ... 2438
Marcus, S K ... 2244*
Marczylo, E L ... 3001*
Marescotti, D ... 1106
Marimuthu, S ... 3115
Marini, C ... 1382
Marin-Kuan, M ... 1866, 2431
Marinovich, M ... 2861
Markell, L K ... 1955*, 3064, 3072
Markham, D A ... 1138
Markham, P M ... 2441, 2442
Markov, D A ... 3541*
Markovic, M ... 2451
Marone, P A ... 3011
Marques, M ... 1146
Marquina Castillo, B ... 1978
Marraffino, S ... 2482
Marroni, D ... 1422
Marroquin Cardona, A G ... 2283
Marroquin-Cardona, A G ... 2269
Marshall, B ... 2840
Marshall, M ... 2247*
Marshall, N ... 2862
Marshall, N B ... 2866
Marshall, S ... 2752
Marshall, V A ... 2893
Marshburn, J ... 3498
Martell, E ... 1956*
Martens, M ... 2130
Martikainen, M-V ... 1851*
Martin, C ... 1228, 2007, 2477
Martin, E ... 1259, 1262, 2654*, 2749, 3199, 3211
Martin, F ... 1106, 3035, 3036, 3401, 3402
Martin, M ... 1075, 1151, 1803, 2625*
Martin, M T ... 2637, 3437
Martinez, I ... 3065
Martinez, S ... 1235
Martínez, M ... 2885
Martínez, M A ... 2885
Martínez-Larrañaga, M R ... 2885
Marty, M ... 2030, 3540
Marty, S ... 1891
Martyniuk, C ... 2635, 2952
Martyniuk, C J ... 2344, 2412*
Maru, J ... 3482
Marukian, S ... 2006
Marumoto, M ... 1583
Marusczyk, J ... 2709
Maruya, K ... 2955
Marvel, S ... 2636*
Marx, T ... 1458*, 1459
Marx, U ... 2187
Marzocchi-Machado, C M ... 2593
Maschmeyer, I ... 2187*
Maser, T P ... 1344
Masereeuw, R ... 3113
Massarsky, A ... 3038*, 3284
Massey, T ... 3277
Massey, V L ... 2211
Masten, S ... 1451
Masten, S A ... 2864
Mastovich, J ... 2773
Masuda-Herrera, M ... 1991*
Masumori, S ... 2063
Masumura, K ... 1389*
Masuno, K ... 3249
Masuoka, H C ... 1958
Mata Espinosa, D ... 1978
Matheson, J ... 2202
Mathias, N ... 3440
Mathieu, G ... 1806
Mathis, C ... 1106, 3036
Mathis, M ... 3298
Mathur, S ... 1073
Matsuda, N ... 1826
Matsui, M ... 3218
Matsumoto, A ... 1393
Matsumoto, N ... 1931
Matsunari, N ... 2471
Matsuoka, Y ... 1113
Matsushima, S ... 3136
Matsuura, M ... 1604
Matsuyama, K ... 1626
Matsuzaki, H ... 1354*, 1355, 1356, 1357, 1358, 2784
Matter, H ... 2647
Mattes, W B ... 1318, 2488*
Matthews, J ... 1103
Mattia, A ... 2279
Mattie, D R ... 1204, 1434*
Mattingly, C J ... 1086*, 1176, 2645
Mattix, M E ... 3276
Maturu, P ... 3158*

Maurissen, J P ... 1179
Mauzy, C ... 3216
Mauzy, C A ... 1094
Mawn, M ... 1124
Maxwell, G ... 2646*
Maxwell, L K ... 3108
Mayer, A M ... 1924
Mayer-Proschel, M ... 1841
Mayes, R S ... 2228
Mayeux, J ... 2874
Mayfield, D B ... 2270
Mayhan, W G ... 2384
Mayhew, J ... 2997
Maynard, S K ... 2927
Maynor, T ... 3177
Mays, C A ... 1521, 1524, 1895*
Mazur, C ... 1885
Mazur, C S ... 3413
Mbemi, A T ... 1327*
McAleer, C W ... 1228, 2007, 2477, 2851
McBride, C R ... 2817, 3238
McCabe, M J ... 1349
McCain, W C ... 1434
McCallister, M M ... 1213
McCanlies, E C ... 1278
McCant, D D ... 2994*
McCarthy, C E ... 1283*, 3047
McCartney, M B ... 3544*
McCaskill, M ... 1307*, 2968*
McCawley, L J ... 3541
McCawley, M ... 2773, 2962, 3248, 3379*
McClain, C ... 1901, 1950, 1976
McClain, C J ... 1961
McClain, S ... 1350
McClanahan, M ... 2145
McClelland, R ... 1673, 3544
McClure, C ... 2782
McClure, P ... 1436
McClure, P R ... 2699*, 2702
McCluskey, J ... 2663, 2854, 3174
McConnaghy, E M ... 3397
McCracken, J ... 2134
McCubbin, F M ... 2254
McCullough, S ... 2489, 3220
McCullough, S D ... 1429, 2490, 3215*
McDaniel, K L ... 1215
McDaniel, L ... 2397
McDermott, T R ... 1252
McDiarmid, M ... 1561
McDonald, J ... 1114, 2258, 2261, 3395
McDonald, J D ... 1304, 2100, 3137, 3444
McDowell, M ... 1638
McDuffie, J E ... 1653*, 1671*, 1672, 3476
McDyre, B C ... 2728*, 3468, 3527
McElroy, C ... 2125*
McElroy, P B ... 1209*, 2406
McEuen, K ... 1091*
McFadden, J ... 2208
McGarrigle, B ... 2902
McGarry, K G ... 2404
McGaw, L J ... 1495
McGee, J ... 1302
McGee, J R ... 2765
McGee, M ... 1065*, 1070, 2135
McGeehan, J K ... 3061
McGill, M ... 2491
McGill, M R ... 1234*, 1971, 2319, 3178
McGlothan, J ... 2353
McGlothan, J L ... 1590, 1591
McGovern, K A ... 2903*
McGovern, T ... 2566*
McGowan, K J ... 1601
McIlwain, E ... 2846
McIntosh, L ... 3041
McInturf, S M ... 1201, 1205
McIntyre, B ... 2550, 3261
McKarns, S ... 2877*, 2878*
McKee, R H ... 1765*
McKenzie, C M ... 1984
McKeon, M ... 1395, 2019
McKernan, L ... 2162
McKim, J M ... 2188, 2589, 3009
McKinney, W ... 1199, 1284, 1313, 1921, 2346, 2538, 2830, 3485
McKinnon, J ... 2831, 2991
McLamb, W ... 2851
McLanahan, E D ... 2235*
McLeod, H L ... 2751
McLoughlin, C E ... 1515, 3496
McMahen, R ... 3539
McMahon, C ... 2826, 2829
McMahon, S ... 1050*
McMartin, K ... 3112, 3116
McMaster, M T ... 3236
McMillian, M ... 1998, 3557
McMinimy, R A ... 2392*
McMullen, P ... 2422
McMullen, P D ... 1084, 2039, 2432*
McNamee, P ... 1616
McNeil, K ... 1402
McNeil, S E ... 1525
McNerney, M E ... 3256
McNutt, P ... 1602
McNutt, P M ... 3063*
McPherson, S ... 2479, 3098
McQueen, C A ... 2213
Meade, B J ... 2862, 2866
Meade, M ... 2734*
Meade, M L ... 2119, 2887
Meador, J P ... 2936
Meador, K J ... 1245
Mecklenburg, L ... 3005
Medeiros, M ... 3134
Medgyesi, D ... 2259
Medina, S ... 1350*
Medina-Esparza, L ... 3021
Medina-Esparza, L E ... 2271
Medvedeva, N ... 1247
Medvedovic, M ... 1537
Meecham, K ... 3042, 3101
Meek, B ... 3410
Meek, E C ... 2091, 2109, 2725*
Meek, M B ... 1011*
Meeks, B ... 1883
Megehee, E ... 1380
Megill, J R ... 1668
Megrath, B ... 3099
Megyesi, J ... 2496, 3115
Mehendale, A ... 1646
Mehendale, H M ... 1964
Mehennaoui, K ... 1511, 2805
Mehinagic, D ... 1136, 2234, 3462*
Mehinto, A ... 2955
Mehling, A ... 2200
Mehrpouya-Bahrami, P ... 1898*
Mehta, J ... 2470
Mehta, R ... 3028*
Mehta, S ... 2148
Mei, N ... 1388, 2592*
Meier, M ... 1448
Meighan, T ... 1289
Meinl, T ... 2656
Meireles, G ... 1408
Meisner, M ... 1800*
Melber, A ... 2107
Melching-Kollmuss, S ... 2068
Melendez, E ... 1088
Melia, J ... 1436, 2708*
Mellado-García, P ... 3023
Mellard, D ... 2235
Mellen, N ... 2833
Mellert, W ... 1115, 2463, 3054, 3057*, 3058, 3536
Melliti, K ... 3101
Mellon, R D ... 1643
Mellor, C ... 2656
Melnikov, F ... 2323, 2324
Melnyk, S B ... 1196
Meloni, M ... 1614
Memon, N ... 3235
Mendelev, I ... 2618
Mendenhall, B A ... 2096*, 2127
Mendes, O ... 3011
Mendez, L B ... 1383, 2255*, 2256
Mendez, M ... 1261*
Mendez, W ... 2168
Mendez-Hernandez, A ... 1255
Mendoza, A ... 1894
Mendoza-Rodríguez, C A ... 3229
Mendrick, D L ... 1110
Menegola, E ... 3279, 3280*
Menezes, C M ... 1933*, 2822
Meng, Q ... 3268
Meng, R ... 1671
Menges, C W ... 2047
Mera, Y ... 2033
Merah, F ... 1415, 1418*
Mercadante, A Z ... 2593
Mercado, G ... 2943*
Mercado-Garcia, A ... 1432, 3204
Mercer, A ... 2412, 2952
Mercer, K ... 2025
Mercer, R ... 2538, 2773, 2800, 3485
Mercer, R R ... 3488, 3496
Merchant, M L ... 2211
Meredith, A N ... 3486
Meredith, C ... 2664, 3031, 3040
Merema, M ... 1997
Merrick, B A ... 3177*
Merrill, D ... 3011
Merrill, E A ... 1204*
Merry, G ... 3073
Mertens, J ... 1930
Mertens-Talcott, S U ... 2593
Merwin, S ... 2407
Mesaros, C ... 1312*, 2317
Messner, S ... 1669, 2000*, 2173, 3460
Metea, C ... 1899
Metodieva, M ... 2890*
Metruccio, F ... 3279, 3280
Metz, R ... 3392
Metz, T O ... 1136
Meulenbelt, J ... 1157
Mewes, K R ... 1616
Meyer, A E ... 3418
Meyer, B ... 1615
Meyer, J ... 1403, 3278
Meyer, J N ... 1028*, 1401, 1848, 2369, 3077, 3284
Meyer, S A ... 1439, 1490, 1491, 3454
Meyerhoefer, B ... 1060
Meyers, J L ... 1368*
Mezaki, M ... 2313
Mezei, G ... 2979
Mezrich, J D ... 1945
Miadzvedskaya, L ... 3486
Miah, M R ... 2357
Miao, L ... 3125
Michail, K ... 2321
Michalovicz, L T ... 1211, 1212*, 1214, 2918
Michel, K ... 2544
Michelotti, G ... 1902
Michelotti, G A ... 1920, 1949
Mickey, K ... 3341
Middleton, A ... 2212, 2596
Mielke, H ... 1544*
Mielke, P ... 3532
Miere, D ... 2276
Miguel, S ... 2622
Mihalchik, A ... 2538
Mihalchik, A L ... 3492*
Mikamoto, K ... 3186
Mikheev, V ... 3043
Mikkelsen, R ... 1925
Miklasevich, M ... 1201
Miklasevich, M K ... 1205
Mikolajczak, H ... 2470
Milanez, S ... 2145*
Milano, S ... 2835*, 2837*
Milchak, L ... 2189*, 2659
Millar, M ... 2455
Millard, D C ... 1745*
Millard, P J ... 1798
Miller, C ... 1559, 1560, 1618*, 2058
Miller, C N ... 3195*
Miller, D ... 1066, 2937
Miller, D B ... 1069*, 1211, 1212, 1214, 2135, 2336, 2824, 2918
Miller, G ... 2105
Miller, G P ... 3155
Miller, G W ... 1191*, 1892, 3537
Miller, J F ... 2473
Miller, K ... 1074, 1090
Miller, L ... 3261
Miller, M ... 1871
Miller, M M ... 1867*, 3418*
Miller, P ... 2877, 2878
Miller, R A ... 2714
Miller, S ... 3155
Miller-DeGraff, L ... 3498
Milliman, E ... 2995
Mills, L J ... 2933*
Mills, M ... 2323, 2324
Milner, V ... 3101
Min, E ... 2125
Minami, T ... 1931
Minarchick, V C ... 1502, 1520*
Minet, E ... 1107*, 3037, 3552
Mingoia, R T ... 3072
Minnema, D J ... 2411
Minnier, B ... 1400
Minso, J ... 2245
Mirabile, R ... 2849
Miranda Labra, R U ... 1973, 1978
Miranda-Castañeda, C A ... 2271
Mirnics, K ... 1178
Mirowsky, J ... 1071*
Mirsalis, J ... 1626*
Misenheimer, J ... 1543
Mishin, V ... 2097
Mishra, A ... 2762*
Mishra, P ... 2489
Misner, D ... 1638, 1669, 1743, 3481
Misra, R S ... 1903
Mistry, J ... 3529
Mitchell, C ... 2782
Mitchell, D ... 3042
Mitchell, S ... 1735
Mitchell, T ... 2652, 3394*
Mitkus, R J ... 1642
Mittal, L ... 1542, 2137
Miura, N ... 2812*
Miyajima-Tabata, A ... 2776*
Miyamoto, M ... 3522
Miyamoto, N ... 1825*
Miyamoto, Y ... 3522
Miyashita, A ... 2001
Miyawaki, I ... 1239
Miyazawa, M ... 2193, 2196, 2198
Mizukami, S ... 2028*
Mizumachi, H ... 2198
Mizushima, T ... 3102*
Mizuta, Y ... 2806
Mo, Y ... 2804
Moe, B ... 1478*
Moeller, B ... 2086, 2742
Moeller, B C ... 3395, 3444*
Moffat, I D ... 1045
Mohamed, W ... 2387
Mohammed, A K ... 1292
Mohammed, A N ... 1170*, 2414
Mohammed, A T ... 3257
Mohanty, S ... 1997
Mohar, I ... 1940*
Mohareb, F ... 3041
Moland, C L ... 3475
Molina, F ... 1636
Moller, K M ... 1695*
Moller, P ... 2248*
Möller, F J ... 3391
Möller, L ... 2788
Møller, P ... 2808
Møller Laursen, S ... 3242
Mollergues, J ... 1866*, 2431
Molnar, P ... 2851
Monachino, M ... 3552
Mondal, T K ... 1894
Mongan, M ... 3268*
Monick, M M ... 2793
Monks, T J ... 1244*, 1599, 2082, 2380
Monnot, A ... 1264*
Monnot, A D ... 2281
Monroe, W ... 2774
Montano, A ... 1626
Montecillas, R ... 1659
Monteiro-Riviere, N ... 1656
Monteiro-Riviere, N A 1504, 2760, 2766, 3078, 3079*
Montes-Fonseca, S L ... 3491
Montgomery, S ... 3394
Monti, S ... 1799
Montoro Bustos, A ... 1522
Montoya, G ... 3536
Moon, Y ... 1942*
Moore, C ... 1224*
Moore, J ... 2470
Moore, M ... 1388
Moore, M J ... 1158
Moore, M M ... 2653*, 3410
Moore, N ... 2277*, 2671, 2696*, 3085
Moore, R ... 1353
Moore, R R ... 2714
Moore, R W ... 2055*

Moore, S A ... 3042*
Moorthy, B 1492, 3143, 3152, 3154, 3158, 3160, 3306b*, 3311*, 3465
Morales, L D ... 2065
Moran, M ... 2339
Moreau, M ... 2229
Moreaux, J ... 2483
Moreira, P ... 1443
Moreno, F ... 2035
Moreno Olivas, F ... 2781*
Moretto, A ... 3279*, 3280
Morford, L L ... 2564*
Morgan, A G ... 2321*
Morgan, D ... 3220
Morgan, D L ... 2131
Morgan, D S ... 1429, 3215
Morgan, E ... 1883
Morgan, J S ... 3507
Morgan, M M ... 3388
Morgan, P ... 1669
Mori, K ... 3186
Moriguchi, H ... 2001
Morikawa, T ... 1960
Morisbak, E ... 2443
Moritz, W ... 2000
Morpurgo, B ... 2026
Morre, J ... 2079
Morris, A ... 1223*, 2780*, 2834
Morris, A M ... 1558
Morris, J B ... 1295, 2959*
Morris-Schaffer, K ... 1188
Morris-Schaffer, K B ... 1166, 1168*
Morse, M A ... 3097*
Mortensen, A ... 2808
Mortimer, R ... 1409
Mortuza, T ... 2908*
Mortuza, T B ... 3448
Moschini, E ... 2786*
Moscibrocki, C ... 1899
Moscovitz, J E ... 3240*
Mosedale, M ... 3175*, 3181
Moser, J ... 2623*
Moser, V ... 2886*
Moser, V C ... 1215
Moses, E ... 3396
Moses, J ... 1379
Mosher, S ... 1803
Mosig, A ... 1082
Mosley, S ... 2279*
Mosqueda-Tapia, G ... 3223*
Mostafa, A M ... 2825*
Mostafaei, F ... 1552
Mostrag, A ... 2163, 2467
Mostrag-Szlichtyng, A ... 2657, 2709*
Motohashi, H ... 1761*, 2198
Motsinger-Reif, A A ... 2751
Mottino, A D ... 3431
Mourier, E ... 3237
Mousa, S A ... 1227
Moustafa, G G ... 3257
Mowinckel, P ... 3258
Mowla, T ... 2896
Moyano Cires, P V ... 2393*
Moyer, R A ... 2404*
Mruk, R ... 1299
Mueller, L ... 1606*
Mues, S ... 1518
Muhammad, F ... 3511*
Mukerjee, E ... 1153, 2390
Mukerji, P ... 1124
Mukherjee, S ... 1387*
Mukhopadhyay, D ... 2594*
Mukhopadhyay, S ... 2510*
Muku, E ... 1733
Muku, G E ... 2425*
Muldoon Jacobs, K L ... 2475
Müller, G A ... 2949
Müller, M ... 1941
Mulligan, C ... 2899, 3426
Mullin, E ... 2356
Mulliner, D ... 2201
Mullis, L ... 2769
Mummert, L ... 3024
Mummery, C L ... 1830
Mumtaz, M ... 1453*, 2227
Mumy, K ... 3216
Mumy, K L ... 1205, 1434
Mun, G ... 2194
Mundy, W ... 2584*
Mundy, W R ... 1150
Muniz Ortiz, J G ... 2267*
Munoz, A ... 2064, 2072
Munsif, M ... 1655
Muralidhara, S ... 3448
Murayama, N ... 3093
Murbach, T ... 1457
Murdock, N ... 2473
Mürdter, T ... 3543
Muriana, A ... 2456, 3299
Murphy, B ... 1225*
Murphy, C J ... 1608, 1610, 3255
Murphy, K ... 1315
Murphy, L ... 2458*
Murphy, L A ... 2893, 2935
Murphy, R ... 3126
Murphy, S ... 2063
Murphy, S K ... 3077
Murphy, W L ... 3080, 3388
Murr, A S ... 1887, 1888*, 1889
Murrary, E ... 1638
Murray, A ... 1658
Murray, I A ... 2419, 2423, 2425, 2426*
Murray, L ... 1666
Murray, P ... 3129
Murrell, D E ... 2720
Murugesh, D K ... 3428
Musa-Veloso, K ... 2286
Muster, W ... 2641
Mutlu, E ... 1451
Mutzke, E ... 3028
Myden, A J ... 2619
Myers, G J ... 1583
Myers, J ... 1359
Myers, L P ... 3372*
Myhre, O ... 1299
Mylchreest, E ... 3261

N

Na, K ... 1423
Naageshwaran, V ... 3083*, 3239
Naash, M I ... 1032*
Nabb, D ... 2925
Nabb, D L ... 1955, 3072*
Nabekura, T ... 1476*
Nacci, D ... 2937
Naciff, J M ... 1117
Nagai, H ... 2714
Nagai, Y ... 2464
Nagano, K ... 2031
Nagano, M ... 1570*
Naganuma, A ... 1564, 1566
Nagarkatti, M ... 1085, 1292, 1898, 1900, 1902, 1920, 1943, 1949, 3213
Nagarkatti, P ... 1085, 1292, 1898, 1900, 1902, 1920, 1943, 1949, 3213
Nagata, K ... 1156, 3456
Nagata, R ... 1931, 3090, 3095, 3099, 3277
Nagavally, R ... 3124*
Nagavarapu, U ... 3514
Nagumalli, S K ... 1490*, 1491
Nagy, A ... 2719
Nahmias, Y ... 1980
Naidu, R ... 1277
Nair, V ... 1257*
Naisbitt, D ... 2499*
Nakagawa, F ... 2203
Nakagawa, T ... 1979
Nakajima, K ... 1190
Nakamura, A ... 3482
Nakamura, J ... 2745
Nakamura, M ... 1431*, 3093
Nakamura, Y ... 1467, 1899, 2598
Nakamura, Y K ... 1899*
Nakano, A ... 1567
Nakano, T ... 3415
Nakao, M ... 2919, 2920, 2921*
Nakasone, A ... 2077*
Nakata, K ... 3348
Nakayama Wong, L ... 2236, 2675*
Nalge, P ... 1617
Nallasamy, P ... 2329, 2821
Nanba, S ... 2888
Nanduri, B ... 1336
Napoléoni, J-G ... 3531
Narasimha, M K ... 2212
Narasimhan, N ... 1650
Narayan, R ... 2764
Narayan, R J ... 2449, 2451, 3335
Narayanan, B ... 2894
Narayanan, B A ... 2073*
Narayanan, N K ... 2073
Nardelli, L ... 1612, 1613, 1614, 2206
Narimatsu, S ... 1868
Narita, K ... 2203*
Nash, J F ... 1805
Natarajan, K ... 2048
Nath, R V ... 1138
Nathanail, A ... 3008
Natsch, A ... 2196
Nault, R ... 1100, 1103*, 1118, 1954
Nawrot, T ... 1142
Nawrot, T S ... 1854
Nazarenko, D ... 2440
Ndubaku, C ... 1666
Neal, M ... 2374
Nealon, N J ... 3014*
Nechev, S ... 3090
Neely, M ... 1146
Neely, M D ... 2339*
Neft, R E ... 1402*
Negro Silva, L F ... 1555, 3344*
Nehrbass, D ... 2442
Neilan, C ... 2826
Neilan, C L ... 2829*
Neilson, A ... 1798
Nelen, V ... 1142
Nelms, L ... 3467, 3480
Nelms, M ... 1101
Nelson, A ... 1415, 1418
Nelson, A J ... 2332
Nelson, B C ... 1404, 1505, 1522
Nelson, C ... 1543
Nelson, D M ... 2726*, 3091
Nelson, G ... 2352
Nelson, M ... 2273
Nemazee, D ... 3128
Nemoto, S ... 2603
Nepal, M R ... 1424*, 3429
Neri-Gómez, T ... 2916
Neshkova, I ... 1116, 1449
Nesslany, F ... 3487
Netzeva, T ... 2988
Neubauer, N ... 2544
Neujahr, D ... 1529
Neumann, A ... 2831
Neumann, C R ... 2474
Neumann, T ... 1105, 1989, 3110, 3119
Newhouse, K ... 1198
Newland, C M ... 1213
Newton, S A ... 2995
Ng, C W ... 1998
Ng, H ... 1079
Ng, H W ... 1865*, 1870
Ng, R ... 1798
Ngalame, N ... 1834
Ngalame, N O ... 1256, 3339*
Ngozi, U R ... 1549
Ngueta, G ... 2975*
Nguyen, A ... 1372, 1373, 1683, 2450, 2634, 2764*
Nguyen, D ... 1392
Nguyen, D G ... 1959, 1996, 2003
Nguyen, E H ... 3080
Niaz, M S ... 2040
Nicholas, J ... 2811
Nicholas, J N ... 3495*
Nichols, C E ... 2817*
Nichols, H ... 1822
Nichols, J ... 2925
Nichols, R ... 2090*, 2420*, 2426
Nicholson, T ... 1872
Nickel, J ... 2003
Nicoara, O ... 3123
Nicol, C J ... 2083, 3094
Nicolas, C ... 1682
Nicolas, C I ... 3437*
Nicolini, V ... 1644
Nie, J ... 2969
Nie, L ... 1552
Nie, L H ... 1545
Niedzwiecki, A ... 2023
Niehof, M ... 2542, 2710
Niehoff, M ... 1633*, 1639
Niemeijer, M ... 1670, 2291*, 2595
Niemuth, N ... 2104
Niemuth, N A ... 2089
Niermann, S ... 1855
Nieskens, T ... 3113
Niimi, S ... 2776
Niino, N ... 3186
Nijoukubo, D ... 3289
Nimick, M ... 1320
Ning, B ... 2746*, 2747, 3450
Nirogi, R ... 1617
Nishida, H ... 2204*
Nishikawa, A ... 1467
Nishimaki, F ... 2031
Nishimaki-Mogami, T ... 2806
Nishimura, K ... 3136
Nishimura, T ... 2156*
Nishimura, Y ... 1354, 1355, 1356, 1357, 1358, 2784*
Nisr, R B ... 2343*
Nitopi, M ... 1380
Niu, B ... 2434*
Nixon, E ... 3435*
N'jai, A ... 3151
Njenga, M ... 1337
Njoku, D ... 1974
Nkpaa, K W ... 1696*
Nobili, L ... 2741
Nocairi, H ... 2865
Noçairi, H ... 2625
Noel, A ... 1753*
Noerenberg, A ... 1980, 3543*
Nogueira da Costa, A ... 2722*
Noh, K ... 1424, 3429
Nöh, K ... 2338
Nohara, K ... 1263
Nohmi, T ... 1389
Nöldeke, J ... 1645
Nolen, G T ... 3013
Nong, A ... 2229, 2716
Noort, D ... 2117
Nordgren, K K ... 3209*
Norenberg, J ... 1324
Nork, T M ... 1608
Norman, K G ... 2209*
Norona, L M ... 1996*
Northcott, C A ... 2836*
Norton, K ... 1646
Norton, M G ... 1647
Note, R ... 1113, 2007, 2015*, 2182, 2477
Nour, M ... 1596
Novak, M ... 3278
Novick, R ... 2273
Novick, R M ... 1445, 1693*
Novikov, O ... 1732
Novin, S ... 2620
Novotna, B ... 1438
Nowland, W ... 1789*
Nowland, W S ... 1801
Noyes, P D ... 1802
Nugraha, B ... 3557
Nukada, Y ... 2193
Nukaya, M ... 2076
Numano, T ... 2033
Numazawa, S ... 2598
Nunes, N ... 3296
Nuño Lambarri, N ... 1973
Nurkiewicz, T R ... 1520, 2817, 2820, 3238
Nusz, J ... 2692
Nwarache, L N ... 2922*
Nwoha, P U ... 3167*
Nye, M ... 2962*
Nyffeler, J ... 1171*
Nygaard, U C ... 3258
Nyhuis, T J ... 1626
Nylander-French, L ... 2739

O

Oakes, H ... 2720
Oberdörster, G ... 1498
Oberdorster, G ... 1168, 2540
Oberdörster, G ... 2539
Öberg, M ... 2966
Oberhauser, A F ... 2177
Obi, E U ... 1484*
Obis, T ... 2407
Obisesan, A ... 1557
O'Brien, D ... 3520
O'Brien, T ... 1666
Obringer, C ... 3512
Obringer, C M ... 2197

O'Callaghan, J ... 2459
O'Callaghan, J P ... 1211*, 1212, 1214, 2336, 2918
O'Connell, K M ... 3094
O'Connor, G ... 3396
Odawara, A ... 1826
Odin, M ... 2708
Odin, M A ... 2699, 2702*
Odnevall Wallinder, I ... 2788
Odom, J ... 2818, 2819
O'Donald, E ... 1132
O'Donoghue, J L ... 1583
O'Driscoll, C A ... 1945
Odukoya, O O ... 1527, 1547, 1568
Odunola, O A ... 1446*
Odwin-DaCosta, S ... 1084
Oesch, F ... 2043
Oestreich, J ... 1829
Offenbacher, S ... 2977
Ogawa, K ... 1467, 2050, 2598, 2806
Ogimura, E ... 1979*
Ogungbe, V I ... 1327
Ogunleke, T ... 2282*
Ogunrinola, O A ... 1535*
Ogunrinola, O O ... 1249, 1527*, 1535, 1547, 1568
Ogunsakin, O ... 1307, 2968
Oguntola, J ... 1463
Oguztuzun, S ... 2748
Oh, B-T ... 1546
Oh, J-H ... 2724
Oh, S ... 2453
Ohbuchi, M ... 2001
Ohlsson-Andersson, S ... 2737
Ohsawa, T ... 1202
Ohtani, K ... 2812
Ojekale, A B ... 1463*
Okada, A ... 1156
Okada, T ... 3432
Okamura, A ... 1156*
Oke, O ... 2053, 3030
Okerberg, C ... 1238
Okeyo, G ... 1829
Oki, N O ... 1101*
Okoli, C O ... 2282
Okumura, S ... 1604
Ola Davies, O E ... 2954
Oladele, G M ... 2485*
Oladipo, A ... 1549
Olaleye, S B ... 1923
Olavarria, J C ... 1790*
Olayemi, F O ... 1557
Oldach, J ... 3066*, 3516
Olden, K ... 1721b*
Oldendorp, A ... 1928
Oldman, K ... 1669
Oleaga, C ... 2007*, 2477, 2851
Olgun, N S ... 1558*
Oliveira, C C ... 2860
Oliveira, D P ... 2860
Oliveira, G A ... 2860, 3296*
Oliveira, R ... 3296
Oliver, A K ... 3420
Oliver, M ... 1561
Oliveri, A N ... 3284
Olivero, O A ... 2738*
Olivero-Acosta, M ... 1088
Olivero-Verbel, J ... 1548*, 2744, 2946
Olivero-Verbel, J T ... 2904
Olivery, A N ... 1175*
Ollier, K ... 1382
Olopade, J O ... 1557
Olsen, G ... 2240
Olsen, J ... 1927
Olshan, A ... 1259
Olson, G R ... 2799
Olson, J ... 2902
Olson, J R ... 2051, 2401, 2402
Oltramare, C ... 3515
Olugbami, O J ... 1446
Omiecinski, C J ... 2434, 2435
Omiwole, R A ... 3297
Omland, K ... 3087
Omobowale, T O ... 2827
Omogbemi, E D ... 3297
Omotosho, I O ... 2735*
On, D ... 3220
On, D M ... 3215
On, V ... 3550
Onakpa, M M ... 1462*
Ondrušová, K ... 1450
O'Neal, S L ... 1551*
Ong, J ... 1132*, 1559, 1560, 2981
Onianwa, P C ... 1276
Onica, T ... 1454
Oniye, S ... 2932
Ono, A ... 2156
Onoda, A ... 1431, 2802*, 2809
Onoue, S ... 2204
Onunkwor, B O ... 1248, 1547
Onunkwor, O B ... 1249, 1568
Onwordi, C T ... 1276*
Onwordi, T C ... 1568
Onyije, F ... 3167
Opperhuizen, A ... 3015, 3046
Orandle, M ... 1314, 1921, 3488
Orbach, S M ... 1999*
Orbea, L ... 3431
Orchard-Hays, J ... 2615
Ordoñez, P ... 3040
Orie, N N ... 2891*
Orlicky, D ... 2752
Orlicky, D J ... 2126
Orozco-Mercado, H M ... 1383
Orr, M ... 1125, 1529, 2360
Orr, M L ... 2326
Orrantia-Borunda, E ... 3491
Ortega, A ... 1216
Ortega, J F ... 2035*
Ortega, L ... 2401
Ortega, M T ... 3078, 3079
Ortega Torres, L ... 3036
Örtengren, U ... 2445
Ortenzio, J N ... 2765*
Ortiz-Martinez, R ... 2271, 3021
Ortiz-Rodriguez, J ... 1578*
Osada, Y ... 2810, 2816
Osakwue, J A ... 1535
Osborne, C ... 2125
Osgood, R S ... 1444*, 1907
Osimitz, T G ... 3447
Osinski, M ... 2829
Oskarsson, A ... 2597, 2737
Osornio-Vargas, A ... 1906, 2253
Osowski, D B ... 1273*
Ossig, R ... 1518
Ostiguy, C ... 2693
Ostuki, T ... 1356*
Otegbayo, B O ... 3019
Otieno, M ... 1008*, 1668*, 1671, 1672, 1986, 3053, 3476
O'Toole, T ... 1836*, 2134
Otsuki, T ... 1354, 1355, 1357, 1358, 2784
Ott, D K ... 2228
Otuechere, C A ... 1968*
Ouadi, S ... 3035
Ouedraogo, G ... 1113, 2577*, 2625
Overton, E ... 3165
Oviedo, A ... 3400*
Øvrevik, J ... 1299, 1450*
Ovsianikov, A ... 2451
Owens, C ... 2659
Owumi, S E ... 1446
Oyagbemi, A A ... 2827
Oyatorisa, M O ... 1471
Oyewo, E O ... 1277, 3424
Ozaki, K ... 2987
Ozcagli, E ... 2220*
Özden, S ... 3205*
Ozhan, G ... 2785*
Özhan, G ... 2761
Öztas, E ... 2761*

P

Pace, C ... 1266*
Pacheco, B ... 1559, 1560
Pacheco, J ... 2736
Pacheco Shubin, S ... 2670
Packert, G ... 2077
Padilla, S ... 1795, 1803, 2252, 2886
Padilla-Banks, E ... 3231
Padrós, F ... 1806
Paech, F ... 1972*
Page, K E ... 2194*
Pain-Devin, S ... 2805
Paini, A ... 2656
Paisley, B M ... 1108*
Pal, A K ... 1508
Palani, S G ... 1501
Palate, B ... 3269
Palczewska, A ... 2656
Palermo, C M ... 3441
Palm, T ... 2857
Palmai-Pallag, M ... 2249
Palmberg, L ... 1282, 2171*
Palmeira, C M ... 1127
Palmer, B C ... 1503*
Palmer, J A ... 3299*
Pamelard, F ... 1293
Pamies, D ... 1148*, 2340, 3265
Pampohile, S ... 2356
Pan, D ... 1676, 2830
Pan, L ... 2906
Pan, X ... 1815
Panday, A ... 1908
Pandey, M K ... 1328
Pandey, S ... 1617
Pandiri, A R ... 1609
Pandya, M ... 2942
Pang, J ... 2908, 3447, 3448*
Pang, Y ... 1232, 2345
Pangburn, H A ... 2228*
Panko, J ... 2669, 2976*
Panko, J M ... 1864
Panstingel, N ... 1126*, 1938
Pant, K ... 1398*, 2053
Pantano, P ... 2796, 3486
Paoliello, M M ... 1553*
Papale, A ... 2861
Pape, R ... 2381
Papineni, S ... 1891, 2458, 2893*
Paquette, D ... 1208
Paquette, Z ... 2982*
Paradiso, D ... 2125
Parakhia, R ... 3520*
Paredes, A M ... 2799
Parente, C ... 1926
Parfenova, L ... 1392
Parikh, S ... 3554
Paris, M ... 2202, 2990*
Park, B K ... 3129, 3472
Park, C ... 1735
Park, C S ... 3016
Park, H ... 2729
Park, J H ... 2181
Park, J J ... 2181*
Park, J-S ... 2308, 3141
Park, M-S ... 3217
Park, S-H ... 1942
Park, S-M ... 2724
Park, Y ... 2588, 2881, 2889, 3068
Park, Y-I ... 3074
Parker, A ... 2694
Parker, G A ... 3275, 3276
Parker, P ... 3345
Parker, R ... 2459
Parks, A ... 1881
Parks, A K ... 2191
Parks, B ... 2422, 2432, 3017
Parks, J ... 2259
Parman, T ... 1374
Parmar, A ... 3037
Parmentier, C ... 3461
Parry, E ... 3141
Parry, J ... 1658
Parsons, G ... 2782, 3490
Parsons, G N ... 3494, 3497
Partosch, F ... 3081
Parvez, S ... 2996*
Pasco, D S ... 1490
Pasics, I ... 1457, 1458, 1459
Pastor, M ... 2647, 2684
Patel, A ... 3442*
Patel, D ... 1521, 1524, 2942
Patel, K ... 2086, 3024
Patel, L ... 2826, 2829
Patel, M ... 1209, 2406
Patel, M V ... 2190
Patel, N S ... 1643
Patel, P ... 3453
Patel, R ... 2450, 2764, 3024, 3153
Patel, S ... 2190, 3230*
Patel, V ... 1912
Patel, V S ... 1916*
Patisaul, H B ... 1856
Patlewicz, G ... 2161, 2524*, 2528*, 2677
Patlolla, A ... 2794*
Patri, A K ... 2774
Patry, D ... 3028
Patten, C J ... 3059, 3554
Patterson, A ... 2417, 2420
Patterson, A D ... 2426, 2435, 3430
Patterson, J ... 1644
Patterson, T A ... 1240, 1241, 1242
Patti, M E ... 2514*
Patton, K M ... 1308
Paul, G ... 3042
Paul Friedman, K ... 1727*
Paule, M G ... 1240, 1241, 1242, 1840*, 2334, 2335, 2347
Paules, R ... 1095, 2179
Paules, R R ... 2456
Paules, R S ... 3528
Paul-Friedman, K ... 1886
Pauloin, T ... 2865
Pauluhn, J ... 2987
Paustenbach, D ... 1264, 2669
Paustenbach, D J ... 1445, 1690, 1693
Pauwels, C G ... 3046*
Pavkovic, M ... 3123*, 3476
Pavuk, M ... 3170, 3419*
Pawar, R D ... 2874*
Pawlak, E ... 1754*
Pawlak, E A ... 1338
Pawletta, M ... 1675
Pawlisz, A ... 2662, 2998*
Pearce, G ... 1409
Pearce, R ... 2225*, 2604, 2681
Pearson, K ... 1677*
Peck, B ... 3190
Peddada, S D ... 1609
Pedersen, J M ... 2014*, 3464*
Pedersen, K ... 2025
Pedrosa, R P ... 1309
Pedulla, M L ... 1507
Peeters, L E ... 3472
Peffer, R ... 2018
Peffer, R C ... 2411
Pegram, R A ... 1407*, 2231
Pehl-DeSilva, A T ... 2081
Pei, H ... 2479
Peijnenburg, A A ... 1480*
Peitsch, M ... 3029, 3400, 3402
Peitsch, M C ... 1106, 3035, 3036, 3401
Peixe, T ... 1553
Pekkanen, J ... 1851
Pelch, K ... 2550
Pellegrini, K L ... 3138
Pellevoisin, C ... 2199, 3547*
Peltonen, K ... 3008*
Pemp, D ... 1116, 1449*, 3391
Pena, M M O ... 1852
Pendley, C ... 1641
Pendlington, R ... 2646
Pendse, S ... 1084, 3071
Pendse, S N ... 2039, 2432
Peng, H ... 1123*
Peng, S ... 3051
Penman, M ... 1442
Penn, A ... 3212
Pennell, K ... 3537
Pennell, K D ... 1245, 2730
Pennell, P B ... 1245
Pennella, G ... 2740
Penney, R B ... 1122
Penning, T M ... 1663
Pennington, K ... 1254*
Pennington, K L ... 3184
Pennington, M J ... 2930*
Pepping, T ... 1162
Perdew, G ... 3538
Perdew, G H ... 1733, 2417, 2419, 2420, 2423, 2425, 2426
Perdomo, C ... 3396
Pereira, L C ... 1127*, 1408
Pereiro, N ... 2901
Peres, T V ... 2358
Perez, C M ... 2262
Perez, E J N ... 2484*
Peréz, M J ... 1235
Perez Maldonado, I ... 3134
Pérez-Castro, L ... 1577*
Peric, D ... 1106

Perin, O ... 1113
Peris-Sampedro, F ... 2915, 2916*
Perkins, J ... 3417
Perkins, J T ... 1455*
Perkins, M W ... 2130
Perkins, R ... 1870
Perlina, A ... 2631
Permenter, M G ... 1541, 2728, 3468*
Perpetua, M ... 1631
Perrera, A B ... 3345
Perron, M ... 2689*, 2690
Perry, M ... 3106*
Persaud, I ... 2767*
Perschke, S ... 3534*
Pertuiset, C ... 2015, 3549
Perumal Kuppusamy, S ... 2712*
Perusse, D ... 1959
Perveen, Z ... 3212
Pessah, I ... 1193
Pessah, I N ... 1194, 3415, 3422
Peter, E ... 1115
Peter, S ... 2736
Peters, E ... 2463, 3536
Peters, J ... 1720*, 2021
Peters, J M ... 2020, 2024
Petersen, D ... 2157*
Petersen, E J ... 1522
Petersen, S L ... 1186
Peterson, H M ... 1955, 2925*, 3072
Peterson, K E ... 1858, 3204
Peterson, M K ... 2270, 2670*
Peterson, N ... 2923
Peterson, R E ... 1185, 1187, 2055, 3542
Petrash, J M ... 2115, 2116, 2121
Petri, E ... 2945
Petriello, M ... 1455, 2834*, 3417
Petrikovics, I ... 2096, 2127*
Petrochenko, P E ... 2451*, 3335
Petroff, R L ... 3446*
Petrosyan, P ... 1219
Petrucci, D ... 1658*
Petry, T ... 2544
Pfannenbecker, U ... 1616
Pfau, J C ... 2867, 2868
Pfister, J A ... 1461
Pfuhler, S ... 2535*
Phadnis-Moghe, A ... 1736*
Phadnis-Moghe, A S ... 1360
Pham, L ... 2625
Pham, N ... 2030
Pham, N T ... 3540*
Phan, A V ... 1244
Phan, P ... 2396
Phandthong, R ... 3550
Phelka, A D ... 2138*
Philippot, G ... 1236*
Philipson, C ... 1659
Phillip, O ... 2059
Phillips, B ... 3035, 3402*
Phillips, G ... 2188, 3041
Phillips, J ... 1074, 1090
Phillips, K ... 1679, 1680*, 1681, 1682
Phillips, P M ... 1068
Phillips, T D ... 2269, 2283, 3012
Phipps, R P ... 1283, 1294, 1837, 1911, 3047
Phoon, C ... 1846
Phuong, C ... 3509
Pi, J ... 1251*, 1267, 3071
Picard, C ... 1956
Picard De Muller, G ... 1293
Piccirillo, V J ... 2190, 2942
Piccotti, J R ... 1929
Pichardo, S ... 3022, 3023
Piche, D ... 1454
Piché, M-S ... 1926
Pickering, I J ... 1583
Pickles, J ... 2646
Picut, C A ... 1179, 3275, 3276*
Piekos, S ... 2013
Pieper, A A ... 2397
Pierce, J S ... 1300, 1689, 1690, 2139*, 2140
Pieters, R ... 1334
Pieters, R H ... 2873
Pietka-Ottlik, M ... 2891
Pietrobon, P ... 1624
Pietrofesa, R A ... 2047*
Pike, J T ... 3276
Pimentel, R ... 1997
Pincen, H M ... 1983
Pineiro, S ... 3026
Pinkston, C ... 3170
Piroird, C ... 2206*, 2865
Pittman, G ... 3200
Pixley, S K ... 1598
Pizzurro, D M ... 2679*
Plahovinsak, J ... 2104, 3106
Plahovinsak, J L ... 2089*
Planchart, A ... 1176, 2645
Plano, D ... 1328
Plante, I ... 2046, 2057, 3390
Plante, J ... 2619, 2628
Platof, G E ... 2093
Platoff, G ... 2103
Platoff, G E ... 2102, 2404
Platt, N ... 1630
Platt, V ... 2007
Plavicki, J S ... 1185*, 1187, 3542
Pleil, J ... 1279
Ploch, S ... 3532*
Plourde, D ... 1554, 1555, 3344
Plummer, S ... 2455*
Plunkett, J B ... 2468
Plunkett, L ... 2521*
Plunkett, L M ... 2468
Pluta, L ... 3071
Pognan, F ... 1097, 1675, 2630, 3176
Pogribny, I ... 2035, 2084, 2746
Pogribny, I P ... 1059, 2037, 3189, 3193, 3208
Poindexter, K ... 2106*
Poirier, M C ... 2738
Polanska, K ... 2985
Polen, T ... 3378*
Polifka, J ... 2983
Politano, V T ... 3442
Polk, W ... 2756
Pollard, K M ... 2874
Pollenz, R S ... 3347*
Polli, J E ... 2301
Polman, C M ... 1480
Polotsky, V Y ... 1309
Poltoratsky, V ... 1405, 1414, 1428, 1919
Pomeroy-Black, M ... 1378*
Pompon, D ... 3159
Ponce, F ... 1339
Ponnapakkam, S ... 2980*
Ponnusamy, L ... 1385, 2494*
Pontel, L ... 2086
Poole, L G ... 1984, 2211
Pope, C ... 2106
Pope, C N ... 3108
Pope, D ... 3109, 3139
Pope, M ... 1109
Pope-Varsalona, H ... 2690
Popichak, K ... 2337
Popichak, K A ... 2378*
Popoola, T ... 1662
Porceddu, M ... 2015, 3549
Porter, C ... 2428
Porter, D ... 2538, 2773, 3200
Porter, D W ... 2800, 3496
Porter, N A ... 1178
Porter, W ... 3392
Posgai, M ... 2446, 2448
Post, C M ... 1359*
Poteete, C ... 2695
Pothmann, D ... 3487, 3493
Potrich, F B ... 2860
Pottenger, L H ... 2653, 3410
Potter, D ... 1238
Poulin, J ... 1145
Pouliot, M ... 1208, 2841, 2842, 3105
Poulsen, R ... 1845
Pounds, J G ... 1526, 3043
Powell, E ... 1544
Powell, K ... 2856
Powers, L S ... 2793
Powers, M ... 1974
Powers, M J ... 2493
Powrie, R ... 1442
Prajapati, M ... 2798*
Prakash, Y S ... 3039
Pramanik, K ... 2020
Praminick, K ... 2021
Prasad, G L ... 3038
Prasad, P ... 1904*
Prasad, S ... 1621, 3044*
Prats, E ... 1806
Pratt, M ... 1436*
Prediger, M ... 1441, 1922
Pregenzer, J ... 3009
Prell, R ... 1928
Presnell, S C ... 1996
Preston, B ... 1302
Price, A ... 3314*
Price, K D ... 1332
Price, O ... 2215
Price, P ... 1681*, 1682
Price, P S ... 1679
Prieto, A I ... 3022
Prince, L ... 1565*
Pringle, R B ... 2091*
Prior, H ... 2839*
Prissette, M ... 2407
Privett, B ... 3443
Probst, C E ... 3533*
Proctor, D ... 1040*, 1542
Proctor, D M ... 2137, 2237, 2697
Proctor, W ... 1078
Proctor, W R ... 1669, 3481*
Proffitt, S ... 1849
Prophete, C ... 2247
Proscura, E ... 2098
Prot, J-M ... 2851
Prough, R A ... 1962, 3170
Provencher, A ... 1112
Prows, D R ... 1296
Prozialeck, W C ... 3122*
Prueitt, R L ... 2143*
Pruett, S B ... 1336
Prussia, A J ... 2683
Przedborski, S ... 2407
Przygoda, R T ... 2446, 2448*
Puchalski, J ... 1927
Puente-Valenzuela, C O ... 1255*
Puerto, M ... 3022, 3023
Puga, A ... 1537, 1737, 1823, 3268
Pugh, F ... 2434
Pugh, N D ... 1490
Pugsley, M ... 3105
Pulczinski, J C ... 3214
Pulinilkunnil, T ... 2412
Pulliam, C ... 2025*
Pulster, E ... 2663*
Punganuru, S R ... 2032
Purgatto, E ... 2035
Puschner, B ... 1224, 1892
Puteri, A ... 2278
Putt, D ... 1137
Pyrgiotakis, G ... 2855

Q

Qi, X ... 2963
Qian, W-J ... 1509
Qian, Y ... 2538, 3492
Qiao, D ... 1329
Qin, H ... 2763
Qin, S ... 2495
Qin, T ... 2758
Qiu, P ... 2843
Qiu, Y ... 2070, 2973
Qu, W ... 1251, 3150*
Qu, Y ... 1998, 3557
Quemeneur, L ... 1624
Quevedo, C ... 2456*, 3299
Quezada-Tristan, T ... 2271, 3021*
Quinones-Hinojosa, A ... 1148
Quintanilla-Vega, B ... 1305
Quist, E ... 3271*
Quist, E M ... 3241
Quynh, H T ... 1143

R

R, N ... 2596
Raabe, H A ... 2175
Rabiner, R ... 2442
Rabinovitz, I ... 2129
Racette, B A ... 2352
Racine, C R ... 3109*
Ract, J ... 2035
Radwan, A ... 3443
Rafael-Vázquez, L ... 1305*
Rafferty, P ... 1640
Rager, J E ... 2697, 3407, 3445, 3539*
Ragin-Wilson, A ... 2970
Rahman, A ... 1268
Rahman, I ... 3398
Rahman, M M ... 1277
Rahmani, K ... 2296*
Rai, M K ... 2757
Rai, S N ... 3340
Raimundo, R D ... 2855
Rainho, C R ... 2241
Rajagopal, K ... 1611
Rajagopalan, P ... 1999, 2004
Rajagopalan, V ... 2212
Rajamani, S ... 2826
Rajapaksa, K S ... 3481
Rajendran, K ... 2212
Rajoria, R ... 3239*
Raju, J ... 3028
Raldúa, D ... 1806
Ralph, D H ... 2020*
Ramachandran, K ... 3123, 3138, 3555
Ramaiah, L ... 1632*
Ramaiahgari, S ... 2179*, 2294, 2297
Raman, D ... 2212
Ramanathan, G ... 1128
Ramesh, A ... 1213, 1946, 2040
Ramirez, J-M ... 1177
Ramirez, S C ... 1177
Ramirez, T ... 1788, 2200, 2223, 3052, 3054*, 3057, 3058
Ramirez-Ramirez, H A ... 3012
Ramirez-Salinas, G ... 2956
Ramm, S ... 3118*
Ramme, A P ... 2187
Ramos, E ... 2885
Ramos Robles, B ... 1978
Ramsey, J D ... 2106
Rana, J R ... 2942*
Rana, P ... 3467*
Rand, M D ... 1565, 1569
Randell, S H ... 3505
Randhawa, M ... 1265
Randle, D ... 1988, 2009
Randle, D H ... 1990
Rangel-Muñoz, E J ... 2271
Ranjan, A ... 2078*, 2106
Rankin, G ... 3139*
Rankin, G O ... 3109
Rao, G ... 1927
Rapedius, M ... 1829
Rappold, A ... 2243
Rasmuson, J ... 1286
Rasmussen, T ... 2013
Rasoulpour, R ... 1120, 1869, 2458, 2518*
Rasoulpour, R J ... 1057, 1891, 2674, 2893
Rath, M ... 2023
Rathman, J ... 2163, 2467, 2476, 2657*, 2709
Rattan, S ... 1855*
Raufman, J-P ... 2301
Raulli, R ... 2100
Rausch, L J ... 1398
Ravel, G ... 1624*
Ravenscroft, S ... 2010, 2847*
Ravindranath, V ... 3307*
Rawat, S ... 3413
Rawls, J ... 1791
Ray, J ... 3501*
Ray, P ... 2977
Ray, P D ... 3199
Ray, R ... 1323
Raymick, J ... 2334, 2335
Raymond, J ... 1120, 2036
Raza, H ... 1896*
Re, D B ... 2310, 2407*
Read, R ... 1702
Read, R ... 1893
Reamon-Buettner, S M ... 3484*
Reátegui-Zirena, E ... 1385
Reboulet, J ... 3216
Reboulet, J E ... 1205
Recio, L ... 1422*, 2088
Reddy, G ... 1434
Reddy, P ... 1417
Reed, B ... 2103
Reed, R ... 1303
Reese, D H ... 2462

Reese, R ... 1178
Reeve, I ... 2900
Regalia, D ... 2832
Régnier, J-F ... 3487, 3493*
Reichard, J ... 2478
Reid, F ... 2110*, 2111, 2123, 2124
Reif, D M ... 1683, 1800, 2633, 2634, 2636, 2645, 2751, 3364*
Reilly, C ... 1285
Reilly, C A ... 2244, 2251
Reily, M ... 2726
Reiner, R ... 1291
Reinosa, J J ... 2775
Reinsalu, L A ... 1117
Reisfeld, B ... 1369, 3198
Remick, A K ... 3275*, 3276
Remy, M T ... 2397
Ren, J ... 1652, 1657*
Ren, L ... 3466
Ren, X ... 2051, 2969
Renard, B ... 1518
Renfro, J R ... 1221
Renganathan, M ... 2852*
Renquist, B J ... 3345
Renslow, R ... 1136
Reo, N ... 2734
Resager, W ... 1992*
Reverte, I ... 2915, 2916
Reyes, E S ... 2285
Reynaga-Ornelas, L ... 3219
Reynolds, I ... 3355b*
Reynolds, J ... 2646
Reynolds, S ... 2773, 2782*
Rezk, B M ... 2333*
Rhoades-Hamacher, J ... 2708
Rhokad, D ... 2350
Rhomberg, L R ... 2992
Rial, E ... 1806
Riaz Ahmed, K ... 2678, 2719*
Ribeiro, D L ... 1416
Rice, A ... 3498, 3505
Rice, C ... 1285*
Rice, G ... 1436, 1716b*, 1766*
Rice, G P ... 3009
Rice, J R ... 1811*
Rice, K C ... 2347
Rice, P ... 2558*
Richard, A ... 1682, 2627, 3300
Richard, A M ... 1151, 3539
Richard, C ... 3237
Richards, J ... 1066, 1067
Richards, J E ... 1069, 1200, 2135
Richardson, C ... 2736
Richardson, J ... 1953, 2069, 2348, 2400
Richardson, J R ... 1135, 1969, 2371, 2413, 2554*
Richardson, R J ... 2398*
Richardson, V ... 1885*
Richarz, A N ... 2656
Richburg, J H ... 3255
Richert, L ... 3461*
Richey, J ... 2104
Richman, E ... 2337
Richter, I ... 1818, 3056
Ricke, W ... 1872
Ricles, L ... 2453*
Rider, C ... 2294
Rider, C V ... 1451*, 3150
Ridge, L ... 1844*
Riebeling, C ... 1518
Riefke, B ... 3476
Rieth, S ... 1198
Rietjens, I M ... 2548
Riggs, T ... 1627
Riitano, A ... 2332
Riley, R ... 3348
Ring, C L ... 2681*, 3436, 3437
Rinkevich, J P ... 2615
Rinkus, S ... 2900
Rios-Cabanillas, M ... 1574*
Rioux, J ... 2125
Ripp, S ... 2779, 3556
Risby, T ... 1279
Riss, T ... 3553*
Ritacco, G ... 3519*
Ritchie, R ... 2736
Ritter, D ... 3548*
Rittinghaus, S ... 2537
Rittinghausen, S ... 3499
Riu, A ... 1113, 1228, 2007, 2015, 2182*, 2477
Rivas-Arancibia, S ... 2386*
Rivera, E U ... 3339
Rivera-Caraballo, K ... 1736
Rivera-Castillo, H ... 3021
Riviere, J E ... 1504, 3078, 3079, 3511
Rivina, Y ... 2128
Rivkees, S A ... 1845
Rizzo, M ... 1340
Rizzo, M D ... 1341*
Roach, K A ... 1515*, 3496
Roach, M ... 1155
Robaire, B ... 2057
Robbins, C ... 2696
Roberts, D W ... 2644
Roberts, G K ... 3273*
Roberts, J ... 1921, 3028
Roberts, J E ... 1816*
Roberts, J R ... 1284, 1289, 1515, 3489, 3496*
Roberts, R ... 1630*, 2685
Robertson, A ... 1230
Robertson, L ... 1109
Robertson, L W ... 3416, 3420
Robertson, W ... 1423
Robin, G ... 3415
Robin, L P ... 1784*
Robinson, B L ... 2347
Robinson, C ... 3116*
Robinson, J F ... 3141, 3236, 3409*
Robinson, P J ... 1204
Robinson, S ... 2811
Rocca, M ... 1641*
Rocha, J B ... 1817
Rochani, H ... 1685
Roche, B M ... 1625*, 2856
Rochester, J R ... 1859*
Rochet, J-C ... 2372
Rockwell, C ... 1346
Rockwell, C E ... 1344, 1934, 1965
Rodenburg, L ... 1442
Röder, N ... 3525
Rodgers, D ... 3042
Rodgers, T ... 3099
Rodridguez, A ... 1945
Rodrigo, G ... 3400
Rodrigues, A D ... 1610*
Rodrigues, L ... 3296
Rodrigues, M A ... 3533
Rodriguez, A M ... 2130
Rodriguez, L ... 2647
Rodriguez-Martinez, E ... 2386
Roell, K R ... 2751*
Roessger, K ... 2000, 2173
Roethke, T J ... 1301
Rogawski, M ... 1229
Rogers, J ... 2717
Rogers, L K ... 1913*
Rogers, T ... 3090, 3095
Rohan, J G ... 1201, 1205*
Rohlman, D ... 2902
Rohlman, D S ... 2401*, 2402
Rohr, A C ... 2137
Rojanasakul, Y ... 3483
Rojo de la Vega Guinea, M ... 2602*
Rokad, D ... 2351*
Rolle, D ... 1552
Rollins, B ... 3547
Rolls, B A ... 3039*
Rolo, A P ... 1127
Romero, A ... 2885
Romero, E ... 1285
Romero, E G ... 2244
Romero, M F ... 1271
Romo, D ... 1907*
Romoser, A A ... 3012
Rompion, S ... 1665*
Rondelli, C M ... 3149, 3151*
Ronis, M ... 2025
Ronis, M J ... 1874*
Ronnekleiv-Kelly, S M ... 2076
Roof, S ... 2848*, 3531
Roomi, M W ... 2023*
Roomi, N W ... 2023
Rooney, J P ... 2624*
Roper, C ... 1614
Roper, C S ... 1603*
Roper, J ... 3025, 3064
Roponen, M ... 1851
Roque, P J ... 1207*
Rosales Cruz, D P ... 1973
Rosas Lemus, M ... 1973
Rosas-Hernandez, H ... 2347
Rosas-Perez, I ... 1906, 2253
Roseman, J ... 2110, 2111, 2123, 2124
Rosemberg, D B ... 1817
Rosen, M ... 1822*, 2547
Rosen, T ... 3272
Rosenbaum, J T ... 1899
Rosenberg, K ... 3278
Rosenberg, M ... 1084
Rosenfeld, M E ... 1128
Rosengren, R J ... 1320*
Rosenmai, A K ... 2597*, 2737
Rosenspire, A ... 1349*
Rosenthal, F ... 1596
Rosenzweig, B ... 3473, 3474
Ross, J ... 2096, 2856
Ross, J D ... 1745
Ross, M ... 2899
Ross, M K ... 2725
Ross, P ... 2168*, 2169
Ross, S M ... 1867, 1871, 3071
Ross, T M ... 1407
Roth, A ... 1828
Roth, J ... 2356
Roth, R ... 2008, 2497*, 2502*
Roth, S E ... 3175
Roth, T ... 1561
Rothe, H ... 2466, 3512
Rothe, M ... 3045
Rothemund, S ... 2477
Rothenberg, C ... 2980
Rothenberg, S ... 1584*
Rothen-Rutishauser, B ... 1708*
Rothfuss, A ... 1645*
Rothwell, C ... 2107, 2130
Rouach, H ... 2430
Roursgaard, M ... 2248
Rouse, R ... 3473*, 3474
Rousseau-Ralliard, D ... 3237
Rousselle, S ... 3084
Rovedatti, M G ... 2901
Rowland, D ... 2409
Rowlands, J C ... 1736, 1770*, 2422, 2711
Rowley, S ... 1871, 3071
Roy, A K ... 3504
Roy, N ... 2938
Roycroft, J H ... 1308
Royland, J E ... 1200
RS, T-E ... 1494
Rubelli, M ... 1752*
Rubin, A ... 2900*
Rubino, R E ... 2083, 3094
Rubio-Andrade, M ... 1259
Rubio-Armendariz, M-C ... 2278*
Rubitski, E ... 3480
Rudel, R ... 2655*, 3157
Rudisill, C A ... 2615
Rudolph, J ... 1666
Rudraiah, S ... 2013, 3431
Rudy, M ... 1841*
Ruepp, S ... 1674, 1676*
Ruestow, P ... 2978
Ruestow, P S ... 1689, 2139
Ruff, A L ... 1601
Ruger, J ... 1605
Ruhter, M C ... 2615
Ruio-Andrade, M ... 2749
Ruiz, I ... 3083
Ruiz, M ... 3067*
Ruiz, M-J ... 2276*
Ruiz, P ... 2227, 2631*
Ruiz-Ramos, R ... 2325*
Ruiz-Uribe, A J ... 2269
Rukke, H V ... 2444
Rumbeiha, W K ... 1226, 2119*, 2120, 3346
Rumpler, J ... 3439
Runge, F ... 1618
Runge-Morris, M ... 3457
Ruppert, M ... 1060
Rusnak, ScD, S M ... 1691*
Russ, K A ... 1284, 1313*
Russell, A L ... 3454
Russell, P ... 2183
Russell, R ... 1395
Russo, D ... 2626
Rusyn, I ... 1010*, 2711, 2729, 3189, 3190, 3433, 3434
Ruszkiewicz, J A ... 1581*
Rutagarama, P ... 3398
Rutten, J M ... 1231
Ryan, C ... 2195, 2196*
Ryan, C S ... 1946
Ryan, E P ... 1081, 3014
Ryan, K R ... 2131*
Rychlik, K A ... 3214*
Ryde, I T ... 2369, 3077

S

Sa, R C ... 2185*
Saal, F v ... 1872
Saba, W ... 2791
Sabate, J ... 1265
Sabbisetti, V ... 3134
Saber, A T ... 2808
Sable, H J ... 3418
Sabo-Attwood, T ... 2787, 2811, 3495, 3508
Sabolsky, E M ... 1520
Sachanandani, R ... 1794, 1807*
Sacher, O ... 2657, 2709
Sadekar, N ... 1571*
Sadekova, N ... 1646
Sadler, N C ... 2234, 3043*, 3462
Sadowski, N ... 2194, 2209
Safe, S ... 2056, 2066, 2074, 2080, 2309, 2337, 2418
Safe, S H ... 1917, 2044, 2378
Saffari, A ... 1217
Saforo, D ... 2496
Sager, T ... 3488, 3489, 3496
Sager, T M ... 2800*
Sahay, K ... 1423
Saiduddin, M N ... 2264*
Saini, Y ... 1935*
Saito, K ... 2193, 2198*
Saito, M ... 1595
Saitta, K S ... 2370*
Saiz, J ... 2647
Sajja, R K ... 1621*, 3044
Sakaguchi, H ... 2193, 2732
Sakai, A ... 1931*
Sakai, Y ... 1393
Sakaki, H ... 2603
Sakakibara, T ... 1604
Sakamoto, M ... 1583
Sakamuru, S ... 2462
Sakanishi, K ... 2464
Sakkiah, S D ... 1079, 1865, 1870
Sakolish, C ... 3111*
Sakuma, M ... 2198
Sakurai, K ... 3186
Sakurai, T ... 2060, 2810, 3164
Sala Benito, J V ... 2656
Salama, M ... 2387*
Salanga, M C ... 1799*
Salatas, J H ... 2668
Salazar, K D ... 2700*
Salazar, R ... 1384*
Salinas, E ... 1788
Salisbury, J ... 2773
Salley, J T ... 1439*
Salmen, R ... 1289, 2830
Salminen, W F ... 1110
Salva, A ... 1618
Salvador, A A ... 2352
Salvetat, N ... 1636
Samet, J ... 2210
Samet, J M ... 2320
Samir Essa, S ... 3006
Sampat, V ... 1916
Sampson, D A ... 2079
Samuel, P T ... 1946
Samuelsen, J T ... 2443*, 2444, 2445
Samuelson, D J ... 2067
Sanchez, B ... 1114, 1309, 2261, 3204
Sanchez, B N ... 1858
Sánchez, D J ... 2795
Sanchez-Casas, R M ... 2269, 2283
Sánchez-Martín, F J ... 1537
Sánchez-Ramírez, B ... 1261, 1262
Sánchez-Ramírez, B E ... 1493, 3491
Sanders, A P ... 1432*

Sanders, J M ... 2045, 3406
Sanders, M A G ... 2067
Sanderson, J T ... 2044
Sanderson, T ... 1481*, 3393
Sandy, M ... 2030, 3540
Sanford, J C ... 2481*
Sangodele, J O ... 3244*
Sanni, A ... 1133
Sanoshy, K ... 2457
Sanquer, S ... 2743*
Sant, K E ... 3291*, 3292, 3294
Santacruz-Márquez, R ... 3228, 3229*
Santhanam, N ... 1228*
Santiago, N ... 2356
Santirocco, E ... 1614
Santonen, T ... 2142
Santos, A C ... 2399
Santos, J H ... 3065
Santos, J M ... 1137
Santos, N A G ... 2399
Sanz, P ... 3087
Sapiro, J ... 1372
Sapiro, J M ... 2599*
Sapkota, M ... 1280*
Sappington, D R ... 1122
Sarang, S S ... 2036, 2038
Sareen, P ... 1398
Sargeant, A M ... 3097
Sargent, L ... 1701, 2773, 2782, 3488
Sarigiannis, D ... 1698*, 2472*
Sarigiannis, D A ... 2985*
Sarkar, S ... 2022, 2334, 2335*, 2373
Sarma, S N ... 1218*
Sartor, M ... 1089
Sarty, K ... 2412
Sasaki, S ... 1604
Sasek, T W ... 1491
Sasidharan, A ... 2766*
Sasso, A ... 1041*
Sasson, C ... 2474
Sato, K ... 3522
Sato, R ... 1904
Satoh, M ... 1528, 1532, 1534*
Satterwhite, C M ... 1928*
Saudan, J ... 2194
Sauer, U G ... 2544
Saulles, A ... 2148
Saunders, J ... 1261
Saurabh, K ... 2496
Savich, R D ... 1232, 2345
Savidge, M ... 3203
Sawada, K ... 1825
Sawyer, A ... 2440
Sawyer, M E ... 2608
Sayama, A ... 3186
Sayers, B C ... 3025*, 3503
Sayler, G ... 2779, 3556
Sayre, A L ... 1291
Scabilloni, J ... 3488
Scanlan, L D ... 1404*, 1417
Scazziota, A ... 1235
Schaffer, J M ... 3472
Schallmo, M ... 1735
Schaudien, D ... 2537, 3499*
Schaupp, C M ... 1499
Schecter, A ... 1143*, 2733
Schenk, L ... 2966*
Schepky, A ... 2531*, 3512
Scherer, R ... 3337*
Scherzer, M T ... 2496
Schetinger, M R ... 2358*
Scheuerell, C ... 3532
Schierle, J ... 3004
Schiestl, R ... 1392*, 2128
Schildknecht, S ... 2381*
Schilter, B ... 1866, 2431
Schinaman, R C ... 1117
Schisler, M ... 1057
Schladweiler, M ... 1066
Schladweiler, M C ... 1067*, 1068, 1069, 2135, 3195
Schlager, J J ... 1506
Schlechtriem, C ... 2925
Schlenk, D ... 1781*, 2665, 2934, 2941, 2949, 2955, 3290
Schlezinger, J ... 1440*, 1883
Schlink, S ... 3084
Schlosser, A ... 2085
Schmalbach, K ... 1116*, 1449
Schmeda- Hirschmann, G ... 3463
Schmeink, S ... 1157
Schmidt, F ... 2647
Schmidt, J T ... 2344*
Schmidt, N ... 2669
Schmitt, E E ... 3392*
Schmitt, M ... 1645
Schmuck, M R ... 1082*
Schmued, L S ... 2335
Schnackenberg, L K ... 1110*
Schneckenburger, J ... 1518
Schneider, A J ... 2055
Schneider, J ... 2353, 3196*
Schneider, K ... 2519b*
Schneider, L ... 3137
Schneider, M M ... 3094
Schneider, S ... 1252
Schnelle, A ... 3126
Schnellmann, R G ... 2496
Schnepper, M T ... 1228
Schoeny, R ... 2653, 3410
Schoetens, F ... 1653
Schoeters, G ... 1142
Scholle, S ... 2221
Schomaker, S ... 2482*, 3558
Schoonen, M A ... 2254
Schophuizen, C M ... 3113
Schoville, S ... 2923
Schreiver, I ... 3525*
Schrenk, D ... 2591
Schröder, K ... 2710
Schroeder, A ... 2928
Schroeder, J ... 1454
Schroeder2, A ... 2214
Schroth, W ... 3543
Schubauer-Berigan, M ... 3488
Schuchardt, S ... 1510, 2542, 3548
Schuler, L A ... 3388
Schuler, M ... 1060
Schultheis, P ... 2356
Schultz, I ... 1697, 3404*
Schultz, M ... 1340
Schultz, S T ... 1335
Schultz, T W ... 2644
Schulz, M ... 2043
Schumacher, J D ... 1953*
Schunck, W-H ... 3045
Schutt, L ... 1638
Schutte, M G ... 1152
Schwab, C ... 2476, 2709
Schwanke, L ... 3024
Schwanz, H A ... 2447
Schwartz, J ... 1071
Schwartz, T S ... 1816
Schwarze, P E ... 1450
Schwegler-Berry, D ... 1515, 2783, 3488, 3496
Schwotzer, D ... 2542*
Sciaky, D ... 1086
Scialis, R J ... 2316*
Scialli, A R ... 1006*
Scinicariello, F ... 2972*
Sciuto, A ... 2559*
Sciuto, A M ... 1323, 2130
Scollon, E ... 2680*
Scott, C ... 3069
Scott, P K ... 1300*, 1690, 1693
Scott, S ... 2183
Scotto, J ... 2824
Scoville, D K ... 1499
Scruton, K ... 2235
Searfoss, G H ... 1739*
Seaton, M ... 2162
Seaver, B P ... 1361, 1367
Seawright, A G ... 2274*
Sebastián, D ... 1806
Sebbel, J ... 1222
Sebesta, M ... 3545
Seeland, M ... 3081
Seeley, M ... 1692, 2141*
Seely, J C ... 1893
Segal, D ... 1203*, 2984
Segner, H ... 2925
Segura-Medina, P ... 1906, 2253
Sehgal, N ... 1184*
Sehr, A ... 2860
Seidler, F J ... 1163*
Seifalian, A M ... 3334*
Seifikar, H ... 3409
Seiji, S ... 1862
Seisenbaeva, G ... 2171
Sekino, Y ... 1172, 1827, 3263, 3264
Sekowski, J W ... 2107, 2117*
Selaturi, V ... 3526
Selinger, D ... 2605
Selinger, D W ... 2618*
Selwyn, F P ... 3144
Semiu, B ... 1549
SenGupta, S K ... 3094
Sens, D A ... 1257, 1258, 1273, 1531, 3127, 3132
Sens, M A ... 3127
Seo, J ... 1316*, 2890
Seo, J-E ... 2871, 2872*
Sepulveda, M S ... 1792
Sepúlveda, M S ... 2858
Serchi, T ... 1511, 2805
Serra, N ... 2315
Serrano, S ... 1690
Serve, K ... 2868
Sestak, M ... 3162
Seth, R ... 1902
Seth, R K ... 1920*, 1949
Sethi, S ... 1191, 1192, 1193*
Sethupathy, P ... 3211
Seto, Y ... 2204
Setser, J ... 1930
Settivari, R ... 3546*
Settivari, R S ... 2208
Setzer, P B ... 1338
Setzer, R W ... 2681, 3050*, 3437
Setzer, W ... 2225
Seung Mee, L ... 1287
Sewald, K ... 2710
Sewer, A ... 3036, 3036
Sexton, M ... 1625
Seyler, N ... 2199, 3547
Seymour, J ... 2727
Sezerman, O U ... 3205
Sha, L ... 2298
Sha, R ... 3423
Sha, Y ... 1427*
Shack, A ... 1336
Shafarin, J ... 1896
Shafer, T ... 1150
Shafer, T J ... 1149, 1151, 2580*
Shaffer, J ... 2538
Shah, F ... 3382*
Shah, I ... 2161*, 2609, 3188, 3304*
Shah, P ... 3115
Shah, P P ... 2496
Shah, R ... 1074, 1090*, 1725*
Shah, S ... 3485
Shah, U-K ... 2772
Shaheen, H M ... 1431
Shai, J L ... 1433
Shaikh, Z A ... 3342
Shakarjian, M P ... 1173*, 1220
Shalat, S L ... 1135
Shamy, M Y ... 1585
Shan, Y ... 1117*
Shanahan, J ... 2262
Shane, H ... 2862, 2866
Shankar, K ... 1874
Shanker, A ... 1946
Shannahan, J H ... 1502*, 1520, 2767, 3503
Shanno, L ... 1986
Shao, D ... 2120
Shao, J ... 2596
Shao, K ... 2165*
Shao, T ... 1961*
Shapiro, A ... 2165
Shapiro, D ... 1285
Shapiro, S ... 1173
Sharanek, A ... 1970
Sharkey, J ... 3472
Sharma, A ... 2407, 3413
Sharma, A K ... 1328
Sharma, M ... 3506*
Sharma, S ... 1465*
Sharma, T ... 3227
Sharma, V ... 1402
Sharp, C ... 3115*
Sharp, C N ... 1984, 2496
Sharp, J ... 3109
Sharpe, M ... 2491
Sharper, V ... 3260
Shaw, E ... 1402
Shaw, R ... 2333
Shay Hynds, E ... 2669
Shearer, J ... 1351*
Sheehan, D W ... 2175
Sheehan, J ... 3100*
Sheehan, P ... 2661*
Sheets, L P ... 2411*
Sheibani, N ... 3142*
Sheinson, D ... 1078, 3481
Shelton, D ... 1238
Shelton, M ... 1627
Shelton, S D ... 1396
Shen, C-L ... 3018
Shen, J ... 1652, 2644*
Shen, L ... 2323, 2324
Shen, P ... 2881*
Shen, S ... 1562
Shepard, K ... 1422
Shepard, P ... 2655
Shepherd, D L ... 2817
Shepherd, D M ... 1361, 1367
Sher, R ... 2332
Sherf, B ... 1982
Sherman, H ... 1988*, 2009
Sherman, K P ... 2130
Sherr, D ... 1732
Shevchenko, V ... 2173
Shi, J Y ... 2083*
Shi, Q ... 1318*, 2614
Shi, S ... 3479*
Shi, Y ... 1826
Shibutani, M ... 2028
Shih, T-M ... 2095, 2114
Shikanov, A ... 3222
Shilpa, N ... 1119
Shim, J ... 1798*, 2332, 3464
Shimelis, H ... 2070
Shimoda, H ... 3186
Shimomoto, T ... 2745*
Shimpi, P ... 1956
Shin, D Y ... 1287*, 1306
Shin, H ... 2947
Shin, K ... 1233
Shinkai, Y ... 1431, 1582, 2295, 2298, 3451*
Shinoda, S ... 3521
Shinohara, N ... 3482
Shipkova, P ... 2726
Shipkowski, K A ... 3494, 3502, 3503*
Shirakawa, T ... 1156
Shirao, T ... 1172*
Shittu, M ... 2913
Shivashankar, V ... 2618
Shock, S S ... 2668
Shockley, K ... 1451
Shockley, K R ... 1609, 2649*, 3089
Shoda, L ... 2218
Shoda, T ... 3182
Shoeb, M ... 1289*
Shokunbi, O S ... 1535
Shorey, L ... 3246
Shpyleva, S ... 1059*, 2037
Shrestha, S ... 3132*
Shropshire, W ... 1143
Shu, L ... 1812
Shuey, C ... 1132, 2981
Shuler, M L ... 2477
Shull, G ... 2356
Shultz, V ... 3487*
Shurin, M R ... 2803
Shvedova, A A ... 1291*, 2803
Siapco, G ... 1265
Siddens, L ... 3161
Siddens, L K ... 2186
Sie, B ... 2658*
Siedlikowski, M ... 3027
Siegel, E ... 1122
Siegel, P ... 2862
Siegmann, M ... 1986
Siegrist, K ... 2773*, 2782
Sierra-Marquez, J ... 2744
Sierra-Marquez, L ... 2744*
Sierra-Santoyo, A ... 1141*
Sierro, N ... 3035*
Sievers, C ... 2439
Sikka, S C ... 2333
Silbergeld, E ... 2579*
Siler, S ... 2218

Silinski, M A ... 3273
Sills, R C ... 1609
Silva, A L C ... 1553
Silva, C S ... 3470*, 3471
Silva, M ... 2884*
Silva, M J ... 1443
Silvano, J ... 1607
Silverman, J ... 1229, 2410
Silverstein, H ... 2077
Silvestre, R ... 1963
Sim, W-C ... 1474*
Simar, S ... 3487
Simchick, G ... 1159
Sime, P J ... 1283, 1294, 1837, 1911, 3047
Simeonov, A ... 1811
Simic, D ... 1671, 1986*
Simmons, C S ... 1845
Simmons, J E ... 1764*, 2231, 3436
Simmons, S O ... 1407, 1886*, 1888, 1889, 2320
Simon, J ... 3190
Simon, K A ... 3463
Simon, T ... 2677
Simon, T W ... 3362*
Simone, N ... 2890
Simon-Friedt, B ... 1061, 3165*
Simon-Friedt, B R ... 1695
Simonich, M T ... 1121
Simonich, S ... 3285
Simons, J ... 1323, 2130
Simons-Burnett, A ... 1664
Simpkins, J W ... 2917
Simpson, K J ... 3472
Sims, C M ... 1505*
Simutis, F ... 1332
Singer, M ... 1986, 3053
Singh, B ... 3476
Singh, D P ... 2290*
Singh, J ... 1650*, 2024, 2831
Singh, K P ... 1734, 1837*, 2494
Singh, N ... 2373, 2396*, 2772, 2910
Singh, N P ... 3213*
Singh, P ... 2845*
Singh, R ... 2902
Singh, R R ... 1904
Singh, U P ... 3213
Singh, U S ... 2024
Singha, S K ... 1487*
Singhal, A ... 2230*, 2661
Singhal, R ... 1874
Sioen, I ... 1142
Sioutas, C ... 1217
Siow, D ... 2496, 3115
Sipe, G O ... 1180
Sipes, N S ... 2604*, 3383*
Sirenko, O ... 2711
Sisco, M ... 2247, 3248
Siskind, L ... 3115
Siskind, L J ... 2496
Sisler, J D ... 2538*, 3492
Sistare, F ... 1677
Sistare, F D ... 2731
Sitapara, R ... 1916
Sivaraman, L ... 3256*, 3440
Sivasankar, M P ... 2132
Siwakoti, R C ... 2683
Sjodin, A ... 3419
Sjögren, B ... 1944
Skelly, B ... 1684
Skinner, J L ... 1507
Skoczen, S ... 1525
Skoog, S A ... 2449*, 3335*
Skovmand, A ... 2248
Skuba, E ... 2605
Slade, L ... 2412
Slaga, T J ... 2065
Slama, R ... 3237
Slattery, M ... 2307
Slavkovich, V ... 3344
Slavov, S ... 2642
Slezak, B ... 2676
Slikker, W ... 1240, 1241*, 1242, 1840
Slitt, A ... 1372, 1956
Slitt, A L ... 1876
Sloan, D J ... 1673*, 3544
Sloper, D ... 3073
Slotkin, T A ... 1163
Slusser, A ... 3127*
Slusser-Nore, A ... 1273
Sly, E ... 2194
Sly, E A ... 2175
Smeester, L ... 1259, 1262, 3199
Smegal, D ... 2275
Smirnov, A ... 2254
Smirnova, L ... 2185, 2340, 3408
Smit, J ... 1334
Smit, J J ... 2873
Smital, T ... 2945
Smith, B ... 1821
Smith, C ... 2689, 3239
Smith, C M ... 1875*
Smith, C S ... 1308
Smith, C V ... 1177*
Smith, D ... 2736*
Smith, D R ... 2511*
Smith, G J ... 1295*
Smith, J ... 1526, 2234*
Smith, J N ... 1129, 1130, 1131, 3462
Smith, K ... 2361, 2362*
Smith, K J ... 1733*
Smith, L ... 1423, 1671, 1672
Smith, L C ... 3508*
Smith, L L ... 1401*
Smith, M ... 1353
Smith, M E ... 2452
Smith, M V ... 1811
Smith, P B ... 2420, 3430
Smith, R ... 2003, 2125
Smith, R D ... 1265
Smith, S ... 2691
Smith, S A ... 2935
Smolik, C M ... 3086
Snawder, J ... 3377b*
Snawder, J E ... 1686*
Sneed, R ... 1819*
Snelling, S ... 2722
Snider, T ... 2103
Snider, T H ... 2093, 2102
Snippe, N ... 1618
Snook, S ... 1653, 1671, 1672
Snow, S ... 1067, 2135
Snow, S J ... 1066*, 1068, 1069, 1756*
Snyder, A ... 1938*
Snyder, J S ... 2133*
Snyder, N W ... 1312, 2317
Snyder, R ... 3453, 3498
Snyder, R J ... 3505*
Snyder, S ... 1596, 2354, 2355
Snyder, S A ... 1138
Soares, A M ... 1806
Soares, B D ... 1591
Sobolewski, M ... 1167, 1188*
Sobolewski, M E ... 1166
Sobus, J R ... 3539
Sogorb, M A ... 3265
Sohn, E K ... 2940
Solaimani, P ... 1217*
Solano, M ... 1432
Soldatow, V ... 2018*, 3434
Solis-Angeles, S ... 3130*
Solis-Wheeler, M A ... 3281*
Sollome, J J ... 3211*
Solomon, G ... 2030, 3540
Solorio-Rodriguez, A ... 1500
Somji, S ... 1257, 1258, 1273, 1531, 3127, 3132
Sommerhage, F ... 1228
Sommers, C H ... 3024
Sommerville, D R ... 2170
Son, D-S ... 2361
Sonawane, B ... 2717, 2984
Sonee, M ... 1671, 1672, 3476
Song, J-Y ... 3074
Song, M-F ... 3197
Song, P X ... 3204
Song, Y ... 3091
Sonn, K ... 1735
Soper, C ... 1247
Sorenson, C M ... 3142
Soret, S ... 1265
Soroka, C J ... 1966
Soscia, D ... 2390
Soscia, D A ... 1153
Soshilov, A ... 2299
Sosinski, L K ... 1426
Sosna, W ... 2110, 2111, 2123, 2124
Soto-Gutiérrez, A ... 3457
Soukup, S T ... 1116, 1449
South, J ... 1813*
Souza, D O ... 1817
Souza, V ... 1973, 1978
Sovadinova, I ... 2308
Spackman, P ... 1153
Spada, A P ... 1951
Spangler, W S ... 1093
Sparks, N R ... 2311, 3286*
Sparrow, M ... 2773
Spear, W C ... 3155
Specht, A J ... 1545*
Speidel, J T ... 3234*
Spellman, R ... 1060
Spencer, R M ... 1104
Spezia, F ... 1624, 3259
Spiegelman, V S ... 3526
Sping, P ... 3515
Spira, A ... 3396
Spirkl, H-P ... 2647
Spognardi, A-M ... 2441
Sprando, R L ... 2771, 2789
Springer, S ... 1398
Sprowles, J L ... 3418
Spungen, J ... 2275
Squibb, K ... 1561
Sriram, K ... 1199, 2346*
Sriram, S ... 1120
Srivastava, S ... 2078, 2134
St John, N ... 1419
St Louis, R ... 1789, 1801
Staflin, K ... 1078, 1611, 1638
Stallings, J D ... 1270, 1541, 3140
Stamou, M ... 2416*
Stampfl, J ... 2451
Stämpfli, M R ... 3221
Stanam, A ... 1664*
Stancescu, M ... 2851
Stancil, I T ... 2892*
Stanciu, O ... 2276
Stanford, E ... 1732*
Stanko, J P ... 3389*
Stankunas, K ... 3281
Stanley, J R ... 2441, 2442
Stanoi, I R ... 1093
Stansfield, K ... 2353
Stansfield, K H ... 1590, 1591*
Stanton, B ... 2569*
Stanton, B A ... 1253
Stanton, P K ... 1173, 1220, 1590*
Stapleton, H ... 1797
Stapleton, H M ... 2888
Stapleton, P A ... 1520, 2817, 2820, 3238*
Star, A ... 1291
Stark, R ... 2646
Starokozhko, V ... 1997*
Starr, C R ... 2217
Starr, T ... 2086
Staska, L ... 1353, 3276
Stasko, N ... 3443
States, J C ... 2067, 3340
States, V A ... 3340
Stauber, J ... 1293, 1983
Stavitskaya, L ... 1400
Stebbins, K E ... 1298
Stedman, D ... 1789
Stedman, D B ... 1801*
Stedman, D S ... 2551
Stedtfeld, R ... 3351
Steele, B ... 2323, 2324*
Steele, C W ... 3024
Steeves, M ... 2618
Stefaniak, A ... 2780, 3488
Stefaniak, A B ... 1515, 1558, 3496
Stegelmeier, B ... 1992
Stegeman, J J ... 1799, 2122, 3159
Steger-Hartmann, T ... 1675*, 2163, 2684
Stegman, M ... 1195
Stegmüller, S ... 2591
Stehno-Bittel, L ... 3555*
Steidl-Nichols, J ... 1238, 2836
Steinberg, P ... 2016
Steinmetz, F P ... 2656
Stenman, L ... 1124
Stephens, M ... 1037*, 2632
Stephens, M J ... 2461*
Stephens, T ... 1974
Stepp, M W ... 2067*
Stermer, A R ... 3255*
Stern, S T ... 1525*
Sterner, T R ... 1204, 1434
Stethem, C ... 1789, 2551
Stethem, C M ... 1801
Stevenhagen, F ... 1744, 1830
Stevens, G ... 2551
Stevens, J ... 2252*
Stevens, J L ... 1108, 1958
Stevens, M ... 1742*
Stevens, S M ... 1223
Stevens, Z ... 3069
Steves, A N ... 3247*, 3253
Stewart, N ... 1625
Stewart, S ... 3473
Stewart, T ... 2352
Stewart, PhD, S ... 3281
Sthevaan, V ... 1617
Stice, S ... 1159, 2279, 2280*, 3076
Stieffenhofer, A ... 2189
Stiegel, M ... 1279
Stiel, L ... 3394
Stilgenbauer, E ... 2988
Stine, K E ... 1375*
St-Jacques, R ... 3530
Stockmann, C ... 1285
Stoeger, T ... 2808
Stoelzle-Feix, S ... 1745
Stoker, T E ... 1887, 1888, 1889
Stokes, J ... 2869
Stolcpartova, J ... 1438
Stolper, G ... 3516
Stolte, M ... 2647
Stölzle-Feix, S ... 1829
Stone, C ... 2982
Stoopen, G ... 1480
Stout, M D ... 1308
Strasser, J ... 3033, 3034
Stratford, K ... 2838*
Straus, V ... 1115
Strauss, V ... 2463, 3052, 3536
Strazdas, L A ... 2194
Streeter, A J ... 2682*
Streifel, K ... 2395, 2410
Stresser, D M ... 3153*
Stricker-Krongrad, A ... 1237, 3082, 3084, 3088*, 3513
Strickland, J ... 2196, 2202*, 2990
Strickland, J D ... 1151*
Strigun, A ... 3054, 3057, 3058
Striley, C A ... 1686
Strock, C ... 2581*
Strock, C J ... 1155, 1745, 2495*
Strohmaier, K&L R ... 1271
Stromberg, A ... 1455
Struble, E B ... 1647*
Strupp, C ... 2068
Stryker, S ... 2726
Strynar, M ... 3439
Strynar, M J ... 3539
Stuart, C ... 1647
Stubbs, R J ... 1208
Stueckle, T A ... 1496, 2783*
Stull, R ... 2605
Styblo, M ... 1259, 1261, 1262, 3343
Stýblo, M ... 2749
Su, C ... 1676
Su, R ... 3075
Suarez, D ... 2320
Suarez, S C ... 2607*
Suárez, E ... 1216*
Suarez-Martinez, J E ... 1342*
Subramani, S ... 1072
Subramanian, A ... 3387*
Subramanian, K ... 2212, 2596, 3061
Subramanian, V ... 2850*
Suchyta, S ... 3529
Suda, M ... 1406
Suemizu, H ... 3093*
Suen, A A ... 3231*
Suga, M ... 1824*
Sugai, S ... 3182
Sugaya, C ... 2464, 3133
Sugiura, Y ... 2464
Sugiyama, M ... 2471
Suguro, M ... 2033*
Suh, M ... 1542, 1719*, 2137*, 2237, 2697

Sulentic, C ... 1126, 1938
Sulentic, C E W ... 1363
Sullivan, J M ... 1648
Sullivan, K ... 2912
Sultana, R ... 1935
Sultatos, L G ... 2106
Sumant, A V ... 2449
Sumida, K ... 1092
Summers, C ... 1669
Sun, A ... 2622
Sun, H ... 1562, 2064, 2072, 2075, 2247, 2322, 3218, 3338
Sun, J ... 1110, 1657, 2601
Sun, K Y ... 2739*, 2978
Sun, Q ... 2588*, 2881, 2889
Sun, R ... 1910, 1953
Sun, Y ... 2356
Sundar, I ... 3398
Sundararajan, M ... 2996
Sung, K E ... 3388
Sung, T ... 1667
Sunger, N ... 2226
Sunil, V ... 2113, 2118*
Sunkara, M ... 2834
Sunmola, K T ... 1814*
Sunmonu, T O ... 1662
Suntres, Z ... 1975*
Supowit, S ... 2939
Surfraz, M B ... 2619
Suter-Dick, L ... 3113
Sutherland, J J ... 1108
Sutherland, V ... 2549, 2550*
Sutliff, R L ... 1529*
Sutter, A ... 1060
Sutter, T ... 1759*
Suttles, N ... 1627
Sutton, D ... 1609
Suzuki, A ... 2313*, 3168
Suzuki, I ... 1113, 1467*, 1826*, 2598
Suzuki, T ... 1263*
Suzuki, Y ... 2816, 3182
Svendsen, E ... 2980
Svensson, K ... 1432
Svoboda, K K ... 1600
Swaka, G ... 1612
Swartzwelder, S ... 2907
Sweeney, L M ... 1434, 2170*, 2232
Sweeney, M ... 1078
Sweet, S ... 3433, 3434
Swenberg, J ... 1425, 2086, 3369*
Swenberg, J A ... 2742
Swenson, G J ... 2461
Swenson, S ... 1376*, 1377
Swerdlow, R ... 3341
Swinden, J ... 2924
Swinka, B B ... 2474
Sydorskyy, Y ... 3172
Synan, C ... 3107
Szabo, D T ... 3034*
Szarek, J ... 2808
Szeitz, A ... 3411, 3412
Szilagyi, J ... 3350
Szilagyi, J T ... 3233*

T

Ta, C ... 2890
Tabata, K ... 2001
Tabata, M ... 1567
Tack, K ... 2688
Tadagavadi, R ... 1930*, 3107
Taghizadeh, K ... 2742
Tagmount, A ... 2655
Tagmount, M ... 3157
Tai, S ... 1483
Tajima, Y ... 3432
Takahashi, A ... 3182
Takahashi, M ... 1960
Takahashi, Y ... 2732
Takasaki, W ... 1202, 3186
Takasu, S ... 2050
Takeda, K ... 1431, 2802, 2809
Takeda, T ... 1868*, 3267, 3456
Takeyoshi, M ... 2198
Taki, K ... 3249
Tako, E ... 1516, 2781
Tal, T ... 1791*
Talamás-Rohana, P ... 1493
Talbot, P ... 2480, 2638, 3032, 3550
Talcott, S ... 2593
Talhout, R ... 3046
Talikka, M ... 3035
Talleur, M ... 1613
Tam, L M ... 3202*
Tamai, S ... 3186*
Tamura, K ... 3148
Tan, C ... 2221*, 2222, 2238
Tan, J ... 2178
Tan, R ... 1652
Tan, S ... 2620
Tan, W ... 1336*
Tan, Y ... 1831
Tanaka, Y ... 3289
Tancredi, D ... 2409
Taneja, G ... 3465*
Tang, D ... 2646
Tang, H-M ... 3105
Tang, L ... 1134, 1814, 2272*, 2274, 3018, 3204
Tanguay, R ... 1729*, 1796, 1808, 2550, 3285
Tanguay, R L ... 1121, 1800, 1802, 1804, 1805, 1849, 2633, 2636, 2792, 3287, 3538
Tao, G ... 2992
Tao, S ... 2602
Tao, X ... 3272
Tarantino, P ... 3107
Tarkhov, A ... 2163, 2467, 2709
Tarrade, A ... 3237
Tarrant, J ... 1078, 1638, 3481
Tassinari, M ... 3073
Tasso, M J ... 1408
Tate, J ... 3139
Tateno, C ... 3432
Taurin, S ... 1320
Tavcar, R ... 3243*
Taxell, P ... 2142
Taylor, A ... 3490, 3498
Taylor, A J ... 3494, 3497*, 3500, 3502, 3503
Taylor, D P ... 2935
Taylor, M ... 3028, 3030
Taylor, M R ... 1185, 1187, 3542
Taylor, P ... 2403
Taylor, R C ... 1080
Taylor, S ... 2328*
Tchounwou, H M ... 1330
Tchounwou, P ... 2794
Tchounwou, P B ... 1326, 1327, 1330, 1331*
Te, J A ... 2611*
Tebbe, C ... 1402
Teeguarden, J ... 3330*, 3368*
Teeguarden, J G 1136*, 1139, 1140, 1526, 2144
Tegenge, M A ... 1642*
Teissier, S ... 1113, 2007, 2015, 2182, 2477, 2865
Teixeira, J P ... 2775*
Teixeira-Clerc, F ... 3427
Tejeda-Benitez, L P ... 2904*, 2929
Tellez Cruz, A ... 3084
Tellez-Rojo, M M ... 1432, 3204
Temitayo, O ... 1549
Temme, T ... 1082
Tenin, G ... 1844
Tenneson, K ... 1246*
Tentschert, J ... 2541
Teo, S ... 1391, 2019*, 3260
ter Braak, B ... 1670*
Teraoka, H ... 2888, 3289*
Territo, P ... 1587
Terrones, M ... 2773
Tertoolen, L G ... 1830
Testa, J R ... 2047
Tetsuka, K ... 2001*
Teubner, W ... 2200
Teuschler, L ... 1436
Tewari-Singh, N ... 2115, 2116*, 2121, 2126
Thackaberry, E ... 1611
Thakkar, S ... 1091, 2750, 3168, 3187*
Thalody, G ... 1674
Thangirala, S ... 1227
Thatcher, T ... 1837
Thatcher, T H ... 1283, 1911, 3047
Thayer, K ... 1074, 1090
Thebault, S ... 1418
Theodoridis, G ... 2985
Theophilus, E ... 3034
Theophilus, E H ... 3033
Therrien, K ... 2605
Thiede, S N ... 2180*
Thiermann, H ... 2561*
Thirion-Delalande, C ... 3518
Thomas, A ... 1645, 3403
Thomas, A D ... 3012
Thomas, C ... 3026*
Thomas, D ... 1802, 1974*, 3514
Thomas, D D ... 1912, 1916
Thomas, D G ... 1136, 1526*
Thomas, D J ... 1543*
Thomas, H ... 1148, 2849
Thomas, J ... 1736, 3035
Thomas, M ... 3543
Thomas, P Q ... 3226*
Thomas, R ... 1682, 2164, 2422, 2606
Thomas, R S ... 2161, 3017, 3050, 3188, 3436
Thomas, T ... 2114*
Thomas, U ... 1829
Thompson, C ... 1043*, 1542
Thompson, C K ... 2507*
Thompson, C M ... 2697*, 2698
Thompson, D ... 2011, 2017*
Thompson, D E ... 2127
Thompson, E A ... 3502, 3503
Thompson, J ... 1313
Thompson, J A ... 1284, 3489*
Thompson, K ... 1385*, 2632, 3473
Thompson, L C ... 2262, 2263*
Thompson, M A ... 3039
Thompson, T A ... 1324*
Thorne, D ... 3031*, 3037, 3040
Thorne, P S ... 2793, 3425
Thornton, C ... 3283
Thornton, C E ... 3282
Thota, B ... 2212
Thrall, B D ... 1509*, 1526
Thrall, K ... 3095
Thrall, R S ... 1295
Threadgill, D ... 2686, 3433
Thrikawala, S ... 1794*, 1807
Tian, X ... 1425
Tian, Y ... 2304, 2437
Tice, R ... 1095
Tice, R R ... 2456, 2616, 3017
Tichenor, S D ... 2832*
Tidball, A M ... 2379
Tien, L-T ... 1232, 2345*
Tiethof, A ... 2400
Tieu, K ... 2343, 3358*
Tighe, R M ... 1064
Tijani, A S ... 1923*
Tijerina, P ... 1846
Tijerina, P B ... 1847*
Tijernia, P B ... 1165
Tilak, K ... 1738
Tilley, S K ... 1683*
Tillmann, T ... 1510, 2542
Tilly, T ... 3329*
Tilly, T B ... 1506*
Tilton, F ... 1802
Tilton, S C ... 2186*
Timchalk, C ... 1129*, 1130, 1131
Timme-Laragy, A ... 3292
Timme-Laragy, A R ... 3291, 3293, 3294
Tingle, M ... 2828
Tippman, B M ... 2186
Tipps, G ... 2379
Tipps, K G ... 2382*
Tirmenstein, M ... 3477*, 3478, 3479
Titorenko, V ... 1481
Tjalkens, R ... 2337, 2359
Tjalkens, R B ... 2378
Tluczkiewicz, I ... 2163, 2650*
To, A ... 3032
To, K ... 1683, 2634*
Toal, B ... 3405
Tobacyk, J ... 2545*
Todor, A ... 3421
Tohon, H ... 2716
Tohon, H G ... 2229*
Tohyama, C ... 1190
Toimela, T ... 2176, 3062
Tokar, E ... 1451, 2556*
Tokar, E J ... 1256, 1834, 2294, 3339
Tokumoto, M ... 1528, 1532*, 1534
Tollerud, D ... 2804
Tolleson, W ... 2746, 2747
Tolleson, W H ... 3450
Tomkiewicz-Raulet, C ... 2429*, 2743
Tona, A ... 1404, 1417
Toner, F ... 1614
Tong, H ... 1066, 1067, 1070*, 2243
Tong, S-Y ... 2383
Tong, W ... 1072, 1079, 1091, 1865, 1870, 2610, 2750, 3166, 3168, 3183, 3187, 3303*
Tonomura, Y ... 3136
Toot, J ... 3107*
Topinka, J ... 1438*
Topper, L ... 1114, 2261
Topping, V D ... 2771, 2789*
Toraason, M ... 2131
Tornero-Velez, R ... 3437
Tornier, C ... 3547
Torous, D ... 1061, 1410
Torres, C ... 2255
Torres-Rivera, W ... 1383, 2255, 2256
Torres-Sanchez, L E ... 1141
Tort, A ... 3129*
Torvinen, M ... 2857
Toseski, J ... 1686
Totah, R A ... 3163
Totlandsdal, A I ... 1450
Toure, R ... 1880*
Towner, R A ... 1002*
Townsend, T A ... 1396*, 2769
Toyama, T ... 1582
Toyoda, A ... 2471, 3055*
Toyoda, K ... 3182
Toyoda, T ... 1467, 2598, 2806
Toyoda-Hokaiwado, N ... 1389
Toyokawa, K ... 1982
Toyokuni, S ... 3164
Toyooka, T ... 1406
Trampota, J ... 3011
Tran, J ... 1619, 2622
Tran, L ... 2810
Tran, N ... 1785*, 2288, 3293*
Tran, T ... 1626
Tran, V ... 2326, 2730
Trani, D ... 1631
Traore, K ... 2329, 2821
Trask, J ... 2005
Trask, O J ... 3175
Traversa, E ... 2807
Travlos, G ... 3089
Trbojevich, R ... 1514
Treff, N ... 3272
Treskes, P ... 1994
Tressler, J ... 2130
Tressler, J A ... 1323
Treviño-Espinosa, R S ... 2269*
Trindade, E S ... 2860
Trinh, M ... 2108
Tripathi, A K ... 2910
Trippier, P C ... 1663
Trisdale, S K ... 3153
Trivedi, K ... 3029, 3552
Trivedi, P ... 3128*
Tröger, R ... 2737
Trombetta, L ... 2911, 2950
Trombetta, L D ... 1571, 2388, 2903
Tron, A ... 2048
Troncy, E ... 2842, 3105
Troth, K ... 1654
Trout, K L ... 1507*
Troutman, J ... 3318*
Troutman, J A ... 2197*
Trube, G ... 1645
Truman, C C ... 1694
Trumble, J T ... 2930
Truong, K M ... 3422*
Truong, L 1121*, 1796, 1800, 1805, 1808, 1849, 2550, 2633, 2636, 2792
Trush, M A ... 2022, 2329
Tryndyak, V ... 2084, 3193*, 3208
Tsaioun, K ... 2632*
Tsamou, M ... 1854
Tseng, C-Y ... 1182, 1345, 1412, 2071, 2383
Tseng, M ... 1078, 3481

Tsirka, S E ... 2254
Tsuboi, I ... 1838
Tsubouchi, T ... 1660
Tsuchiya, T ... 2050
Tsuji, M ... 2965*
Tsukumo, H ... 2471*
Tsunoda, M ... 2464*, 3133
Tsuruoka, S ... 2773
Tsusaki, H ... 3090, 3095
Tuan, R ... 2178
Tubic, A ... 2945
Tucker, D K ... 1856, 1857*
Tucker, L ... 2314
Tudan, C ... 3535
Tuerck, D ... 1644
Tugendreich, S ... 3485
Tunkel, J ... 2708
Tunkel, J L ... 2615
Turesky, R ... 1023*
Turgut Kara, N ... 3205
Turkus, J ... 2008
Turley, A ... 1346, 2168, 3547
Turley, A E ... 1344, 1934*
Turnock, S ... 3518
Turteltaub, K ... 1021*
Tvermoes, B ... 1264, 2273
Twaddle, N C ... 1139
Tyagi, V ... 2910, 3227*
Tyree, C ... 3109, 3139
Tytell, J D ... 3469*
Tzafriri, A R ... 2441

U

U, R ... 3169
Uchendu, C ... 2913
Uchida, M ... 3432*
Uchino, K ... 3482
Ueno, M ... 3136, 3249
Ueno, S ... 3263, 3264
Uetrecht, J ... 1918
Ugbaja, R N ... 1248*, 1249
Uhouse, M A ... 2379
Ukai, A ... 1389
Ukairo, O ... 1073
Ulker, O C ... 2185, 2813
Umbaugh, C ... 1351
Umbright, C ... 1921
Umemura, T ... 2050
Umetsu, A ... 2198
Umezawa, M ... 1431, 2802, 2809*
Umthong, S ... 1346
Unice, K M ... 1689
Unnisa, Z ... 1734*, 1837
Unoki, T ... 1582, 2295, 2298*
Uozumi, T ... 1824
Upham, B L ... 1444, 2308*, 3351
Uppal, K ... 2319, 3537
Uppu, R M ... 1623*, 2314
Uragami, K ... 1355
Urano, K ... 3102
Urbach-Ross, D ... 2676*
Urban, P ... 3159
Urbano, M R ... 1553
Urbisch, D ... 2200*, 2201
Uresti-Rivera, E E ... 1256
Uribe Carvajal, S ... 1973
Uribe-Ramirez, M ... 1500
Urka, I ... 2333
Urrutia-Ortega, I M ... 2790
Usese, A I ... 1277*
Ustundag, A ... 2813
Usuki, F ... 1579, 2289*
Uteng, M ... 3461
Uvsløkk, S ... 2443
Uwai, Y ... 1476
Uwambayinema, F ... 2249

V

Vaara, M ... 3119
Vaara, T ... 3119
Vaidya, V ... 3128, 3134
Vaidya, V S ... 3118, 3123, 3138
Valadares, M ... 3296
Valadares, M C ... 2860
Valcke, M ... 2229, 2716*
Valdes Salgado, R ... 2137
Valdez, C ... 1153
Valdez, J M ... 1200
Valdivia, A G ... 2271*
Valdivia-Flores, A ... 3021
Valdovinos-Flores, C ... 1219
Vale, A ... 2557b*
Valencia, L ... 1514*
Valente, M J ... 1967
Valentine, W M ... 2339
Valentino, S A ... 3237*
Valentovic, M ... 3126*
Valera, I ... 1904
Vallvé, J V ... 2315
van Amsterdam, C ... 1222
van de Lagemaat, D ... 1138
van de Water, B ... 1670, 1757b*, 2002*, 2291, 2293, 2501*, 2595, 3301b*
van den Berg, M ... 1231
Van Den Berg, R ... 2117
van den Brule, S ... 2808
van den Hoek, A ... 1983
Van der Laan, S ... 1636
van der Wal, L ... 2925
van der Water, B ... 2596
van Esch, B ... 2873
Van Gieson, J L ... 1258*
van Kleef, R G ... 1152, 1157
Van Landingham, C ... 2137
Van Ness, K P ... 3110*
van Ravenzwaay, B ... 1115, 1788, 2043, 2192, 2201, 2463, 2537, 2548, 3052, 3054, 3057, 3058*, 3536
van Ravenzwayy, B ... 2223
van Roest, M ... 1334
Van Rompay, A ... 1614
Van Schooten, F-J ... 3046
van Vught, R ... 2184*
van Vugt-Lussenburg, B ... 1866
van Wijk, H ... 1618, 3104*
van Wijngaarden, E ... 1569
Van Winkle, L ... 3458
Van Winkle, L S ... 1288, 3147*
Vanden Heuvel, J ... 1982*
VanDuyn, N M ... 2616*
Vanlandingham, M M ... 1642
Vanosdol, J ... 1402
Vanpoucke, C ... 1854
Vanscheeuwijck, P ... 3029, 3400, 3401
Vanselow, J T ... 2085
Vantangoli, M ... 2174*
Vantangoli, M M ... 1084
Vara, A ... 2828
Varacallo, L ... 1671
Vardaro, R ... 3148
Vardy, A ... 2433*, 2706
Varela, A T I ... 1127
Varghese, A ... 2167, 2169*
Varlet, V ... 3403
Varma, V ... 3013
Varner, K J ... 2331, 2846, 2850
Varsho, B J ... 2136*
Vasiliou, V ... 2752
Vasilyeva, D ... 2077
Vayas, K ... 2113, 2118
Vaysse, P-M ... 1983*
Vazquez, A K ... 2065
Veeramani, B ... 1120
Vega, N ... 2473, 2882
Vega, R ... 1666
Veith, A ... 3154, 3160
Veith, A C ... 3152*
Velalopoulou, A ... 2047
Velisek, L ... 1173, 1220
Veliskova, J ... 1173, 1220
Veljkovic, E ... 3029, 3035, 3402
Vellky, J ... 1872
Velma, V R ... 1326
Velmurugan, K ... 1444
Vemula, S ... 1319
Venancio, V P ... 2593*, 3016
Venkatratnam, A ... 3433, 3434*
Vennemann, A ... 1518
Venosa, A ... 1915*, 2113, 3350
Ver Hoeve, J N ... 1608
Veranth, J ... 1285
Veras, M ... 1846
Verboven, A H ... 1157
Verder-Carlos, M ... 2900
Veress, L ... 2125
Verlohner, A ... 3052*
Verma, K ... 1663*
Verma, R ... 2190*
Verma, V ... 2243
Verner, M-A ... 2975
Vértesi, A ... 1457
Vervoort, J ... 2548
Vessey, J D ... 2628
Vethamanickam, J ... 2596
Vetrano, A ... 1135
Vetrano, A M ... 3233, 3235
Viau, A ... 1628
Viberg, H ... 1236, 2394*
Vickers, A E ... 2486*
Vidal, F A ... 2901
Vieira Peres, T ... 2357*
Vietti, G ... 2808
Vignesh, K S ... 1598
Vigo, M B ... 1235
Vij, P ... 1556*
Vijay, V ... 1111, 2614
Vilanova, E ... 3265*
Vilcassim, M J R ... 1687*
Villarreal-Vega, E E ... 3130
Villeneuve, D ... 2214, 2928
Vimalakanthan, V ... 1518
Vinall, J ... 1614
Vinall, J L ... 1603
Vincelli, N ... 2824, 3103
Vincent, M ... 2694*
Vinci, T ... 2856
Vine, D ... 3416
Viniegra Morales, D ... 1261, 1262
Vinken, P ... 2723
Violin, K B ... 1853
Visconti, N ... 1426, 3546
Visconti, N R ... 2208
Vispute, S G ... 2302*
Vitcheva, V ... 2163, 2467, 2475, 2709
Vitor, A L R ... 2855
Vivanco-Cid, H ... 2325
Vlaming, M L ... 1744, 1830*
Vlasakova, K ... 2731
Vliegenthart, A D ... 1994*, 2727*, 3472*
Vo, P T ... 2203
Vock, E ... 1820, 1821
Voeller, J N ... 3418
Voepel, I ... 3484
Vogel, C F ... 1366*, 1423
Vogel, D ... 2200
Vogel, U ... 2808
Vogl, W ... 1873
Vogt, J ... 1078, 1669, 3481
Vogt, S ... 3416
Voisin, G ... 3196
Vojtisek-Lom, M ... 1438
Volarath, P ... 2467, 2617*
Volkan, J ... 2818, 2819
Vollmer, G ... 3391
Volz, D C ... 1797*
Von Tungeln, L S ... 1642
vonderEmbse, A ... 1842*, 1843
VonHandorf, A ... 1537*
Voong, J ... 2622
Voorhees, J R ... 2397*
Voorspoels, S ... 1142
Vorgrimmler, D ... 2710
Vorhees, C V ... 2368
Vorojeikina, D ... 1569
Voronin, G ... 2328
Voruganti, S ... 1261
Voutchkova, A ... 2324
Voutchkova-Kostal, A ... 2323
Voyer, C ... 1112*
Voytik-Harbin, S ... 2180
Vranic, S ... 2810
Vriend, J ... 3113
Vrijens, K ... 1854*
Vritov, D ... 2836
Vrolijk, M F ... 3015*
Vrolyk, V ... 3269
Vuillaume, G ... 3400, 3402
Vulpe, C ... 2655, 3157
Vulto, P ... 3113

W

Waalkes, M P ... 1256
Wade, M ... 3390
Wade, M J ... 2236, 2675
Wade, T ... 3433, 3434
Wages, P ... 2210
Wages, P A ... 2320
Wagner, A ... 3483
Wagner, V ... 1391
Wagoner, M ... 3069
Wahlang, B ... 1962, 2834, 3417*
Waidyanatha, S ... 1451, 2179, 2294, 2864, 3177, 3273, 3444, 3453
Waigel, S ... 3340
Waikar, S S ... 3123
Waites, R ... 2036
Wako, T ... 2987*
Waldhauer, I ... 1644
Waliszewski, S M ... 2325
Walk, S T ... 1252
Walk, T ... 1115, 2463, 3054, 3057, 3058, 3536
Walker, A ... 3277
Walker, C L ... 1722*
Walker, D ... 2319
Walker, D I ... 1245*, 2730, 3537
Walker, J ... 2605
Walker, L M ... 2311*
Walker, M K ... 3045
Walker, N ... 1767b*
Walker, N J ... 1030b*
Walker, P ... 2010*, 2847
Walker, T ... 2704
Walker, T D ... 2705*
Walker, V ... 2515*
Wallace, K ... 1150
Wallace, K B ... 3209, 3323*
Wallin, H ... 2808
Walling, B E ... 3275, 3276
Wallis, L ... 1519
Wallqvist, A ... 1083, 2611, 3140
Walsh, D M ... 2490*
Walsh, L ... 2260, 2263
Walsh, M T ... 3045
Walter, K M ... 1884, 1892*
Walters, D M ... 3507*
Walz, A ... 2117
Wambaugh, J ... 1679, 1680, 1681, 1682*, 1803, 2225, 2608, 2677, 3439
Wambaugh, J F ... 2604, 2681, 3188, 3436, 3437, 3539
Wamhoff, B ... 2006
Wan, R ... 2804
Wandel, C ... 1645
Wang, B ... 1475, 1479, 3272, 3440, 3523*
Wang, C ... 1240, 1241, 1242*, 1840, 3440
Wang, F ... 1656, 2888, 2944*
Wang, G ... 2304, 2870*, 3191
Wang, H ... 1251, 1267, 1329, 1479, 1479, 1594*, 1916, 2301, 2843
Wang, J ... 1652, 2051, 2870, 2973, 3018*, 3191*, 3268
Wang, J S ... 2274
Wang, J-s ... 1134, 1814, 2272, 3018
Wang, K ... 1111*, 2401, 2402, 2614
Wang, L ... 1496, 2783, 3143, 3152, 3158, 3160
Wang, M ... 1332, 1909*, 1912, 2437*
Wang, N ... 1862, 2997*
Wang, Q ... 1329, 1477, 1737, 1823*
Wang, R ... 3486*
Wang, R-H ... 2796
Wang, R-L ... 1101
Wang, R-S ... 1406*
Wang, S ... 1879*, 2833
Wang, T ... 1746*, 2602, 3065, 3396
Wang, W ... 1482*, 2626*, 3466
Wang, X ... 1117, 1741, 1890, 2307, 2421*, 2906
Wang, Y ... 1228, 1609, 1678, 1797, 2480, 3166*, 3271
Wang, Z ... 1058*, 1456, 1652, 1732, 2041, 2042, 3184
Wang-Hu, J ... 1845
Wanibuchi, H ... 1092, 2031
Ward, A B ... 1877*
Ward, D ... 3109
Ward, E ... 2354, 2355
Ward, E J ... 1596*
Ward, J M ... 2698
Ward, T ... 2181
Ward, W ... 1056

The numerals following the author names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the presenter.

Wareing, B ... 2201*
Warheit, D ... 2544
Warheit, D B ... 2543*
Waritimi, G E ... 3167
Warmka, J ... 2189
Warner, R ... 2482, 3558
Warren, M ... 2686
Warren, S ... 2266
Warren, S H ... 1448
Waseem, S ... 2385
Washington, M K ... 2040
Wassink, B ... 2008
Watanabe, E ... 2810, 2816
Watanabe, K ... 1660*
Watanabe, S ... 3099*
Watanabe, T ... 3148
Waters, K ... 3161
Waters, K M ... 1802
Waters, S M ... 1955
Watford, S ... 1075*
Watkins, A ... 2172
Watkins, P B ... 2218, 3175, 3181, 3476
Watson, A T ... 2892, 3288*
Watson, C Y ... 1508
Watson, G E ... 1583
Watson, S ... 3453
Watt, E D ... 1886
Watt, J ... 1440, 1883*
Watters, G ... 1325
Watts, F ... 2840*
Weatherly, L M ... 1798, 2332*
Weaver, R ... 1630
Weaver, R J ... 1970*
Webb, D J ... 1994, 2727, 3472
Webb, S J ... 2650
Weber, E ... 1105
Weber, E J ... 3110, 3119*
Weber, G J ... 1792
Weber, L ... 3010
Weber, R ... 2243
Weber, S ... 1644
Weber, T J ... 1129, 1130*, 1131
Weber, W M ... 2100*
Webster, T ... 1440
Webster, T F ... 1437*
Weeks, J ... 2925
Weemhoff, J ... 1981, 3092
Weemhoff, J L ... 3178*
Wegwu, M O ... 1696
Wehmas, L C ... 1804*
Wei, C ... 1994
Wei, F ... 1193
Wei, H ... 2852
Wei, L ... 1809
Wei, L-X ... 1572
Wei, Q ... 1589
Wei, X ... 1117
Wei, Y ... 1147*, 2961
Wei, Z ... 3342*
Wei*, L ... 1597*
Weiberger, B I ... 3235
Weihermann, A C ... 2474
Weimer, J ... 1181*
Weiner, J ... 1735
Weinstock, D ... 1640
Weis, H ... 1208
Weisman, S M ... 3011
Weisskopf, M ... 1545
Weissmann, D ... 1636*
Weiwu, J ... 3143
Wek, R C ... 1958
Welch, J B ... 1603
Welch, K D ... 1461*
Weldon, B ... 2181
Weldon, B A ... 1498*
Welker, T ... 2605
Welling, A ... 3008
Wells, E ... 1552
Wells, M M ... 1394
Welsh, C J ... 2683
Weltje, L ... 2927
Wen, X ... 1969*, 2371, 2721, 3272
Wendler, C C ... 1845
Weng, L ... 2317*
Werner, C ... 2706*
Werre, S R ... 1659
Wertman, J ... 3034
Wertman, J M ... 3033*
Wesselkamper, S ... 2164, 2708
Wesselkamper, S C ... 2159
Wessinger, W D ... 1196
West, B ... 1269
West, K ... 3198
Westerhoff, P ... 1702*
Westerink, R H ... 1152, 1154, 1157, 1231*
Westmoreland, C ... 3051
Weston, A ... 1278*, 3443
Westrup, U ... 3242
Wetmore, B A ... 2224, 2604, 3017*, 3437
Wetter, J ... 1605
Wexler, P ... 1386*
Wezalis, S ... 3064*
Wheeler, E K ... 1153, 2390
Wheeler, J R ... 2927
Wheeler, J S ... 2970
Whelan, M P ... 2536*
Whelton, A J ... 1793
White, A ... 2212, 2596, 3051, 3071
White, C ... 2125
White, C A ... 2897, 2908, 3447, 3448
White, C C ... 1499*
White, C W ... 2126
White, H J ... 1164
White, K ... 1324, 1353
White, K M ... 1063*
White, R ... 1676
Whitley, E M ... 1226, 2119, 2120
Whittaker, C ... 2162*
Whittaker, M ... 2465
Whittaker, M H ... 3523
Whittemore, S L ... 3162*
Whitten, D ... 2308
Wiberg, K ... 2737
Wickliffe, J ... 1053*, 1061, 1272, 1447, 1550, 1718*, 3165
Wickliffe, J K ... 1695
Wicks, J ... 3084
Wiegers, J ... 1086
Wiegers, T C ... 1086
Wielecosseles, R ... 2474
Wiemann, M ... 1518
Wiench, K ... 2063, 2544
Wiese, T ... 2652, 3394
Wiesenfeld, P L ... 3049
Wiesner, M ... 1703*
Wiest, E F ... 3045*
Wignall, J ... 2167
Wijeyesakere, S ... 2398
Wijnolts, F M ... 1152, 1154, 1157
Wikoff, D ... 3407*, 3445
Wilbert, M ... 2605, 2618
Wilbur, D ... 2689
Wilbur, M ... 2900
Wilczak, J ... 1299
Wilde, S ... 1060
Wilder, J ... 3535
Wilder, L ... 2235
Wilhelm, C ... 2103*
Wilichinsky, M ... 2826, 2829
Wilke, M ... 2442
Wilkerson, C ... 2308
Wilkey, D W ... 2211
Wilkins, R C ... 3533
Wilkinson, J ... 2924*
Will, Y ... 1029*, 2575*
Willett, C ... 2632, 3003*
Willett, K ... 1779*
Willett, K L ... 2513*, 3283
Willett*, K L ... 3282
Williams, A ... 1682
Williams, A J ... 2627*, 3539
Williams, C ... 3101
Williams, C J ... 3231
Williams, D ... 3161*, 3246
Williams, D E ... 2079
Williams, D P ... 1669*
Williams, G M ... 1820*, 1821
Williams, J P ... 1290, 1294, 1903
Williams, K ... 1514, 3026
Williams, L M ... 3293, 3294, 3353*
Williams, M ... 3032*, 3087
Williams, M T ... 2368
Williams, P ... 1271, 1924
Williams, R V ... 1399, 2629
Williams, R W ... 2336
Williams, S ... 2323, 2324
Williams, S J ... 3555
Willis, M S ... 3322b*
Willoughby, J A ... 1615*
Wills, R W ... 2725
Willson, G A ... 2714*
Willy, J A ... 1739, 1958*
Wilmer, M J ... 3113*
Wilson, D ... 2226
Wilson, D M ... 1706*, 1881, 2191, 2208
Wilson, J Y ... 3159
Wilson, M ... 1061*, 1550, 1695, 2711, 3165
Wilson, S ... 2174
Wilson, S H ... 1390
Wilson, V ... 1635
Wilson, V S ... 2549
Wilt, N ... 2194
Winans, B ... 1359, 1445*, 1693
Winans, B N ... 1368
Winchester, M R ... 1522
Windebank, S ... 2596
Windham, K ... 2314
Wing, C ... 1225
Wingard, C J ... 1064, 2818*, 2819, 3507
Wink, S ... 1670, 2002, 2293, 2595
Winkler, R ... 1500
Winlow, P ... 2722
Winnischofer, S M ... 2860
Winter, M ... 2221
Winters, B R ... 1279*
Wirbisky, S E ... 1792*, 2946
Wirgin, I ... 2938
Wirnitzer, U ... 3104
Wise, C F ... 1539
Wise, J ... 2376*, 3421
Wise, J P ... 1042*, 1539, 2926
Wise, S S ... 1539
Wiszniewski, L ... 3552
Witek, R ... 1974
Witek, R M ... 2493
Witek, R P ... 2493
Witt, K ... 2131
Witters, H ... 2632
Wiwatratana, D ... 1576, 1577
Wnek, S ... 2146
Wohlleben, W ... 1699b*, 2544
Wohlman, I M ... 2094
Woillard, J-B ... 1146
Wolansky, M J ... 2901*
Wolf, A ... 3176*, 3461
Wolf, C J ... 2172
Wolf, K ... 2005
Wolf, K K ... 3175, 3181
Wolfarth, M ... 2538, 2800, 3496
Wolfe, M S ... 2864
Wolff, A ... 1997
Wolfgang, T ... 3065
Wolfraim, L ... 3087
Wolfson, J ... 2999
Wolt, J D ... 3346*
Won, S S ... 1472*
Wong, B ... 2130*
Wong, B A ... 1434
Wong, C ... 1168
Wong, E L ... 1180*
Wong, E T ... 3029*, 3401
Wong, H ... 1638
Wong, P ... 1423
Wong, Y ... 3202
Wongtrakool, C ... 1529
Wood, C ... 1065, 1791, 3439
Wood, C E ... 1056*, 2087
Wood, D M ... 3472
Wood, R ... 1872
Woodall, G M ... 2713*
Woodruff, T J ... 3141
Woods, B ... 1452
Woods, L ... 2867
Woolbright, B ... 2491*
Woolbright, B L ... 3092, 3178
Wooten, K ... 1385
Worcester, C ... 3014
Workman, T ... 1498, 2181
Worlein, J ... 3278
Worley, R ... 2235
Worley, R R ... 2239*
Wormser, U ... 2098*
Worth, A ... 2163, 2476
Worth, A P ... 2475, 2656*
Worthington, C D ... 3507
Woychik, R ... 3065*
Wren, M ... 1135*
Wright, A T ... 2234, 3043, 3462
Wright, J ... 1844, 2455
Wright, P C ... 2763
Wright, R ... 2632
Wright, R O ... 1432
Wronski, S ... 1941
Wrubel, J J ... 1893
Wu, C ... 2963
Wu, F ... 1562, 2064, 2072
Wu, G ... 2437
Wu, H ... 2481, 2600, 2969, 3147, 3467, 3480
Wu, J ... 1521, 1524, 2437
Wu, K C ... 3053*
Wu, L ... 1091
Wu, Q ... 1572, 2041
Wu, T H ... 1554*, 1555
Wu, W ... 1405*, 1919, 2060, 2329, 2810
Wu, X ... 1267, 1671, 3076*
Wu, Y ... 1676, 3455
Wuersch, K ... 2605
Wurpel, J ... 2330
Wusu, A D ... 1249, 1527, 1547, 1568*
Wyatt, T A ... 1280
Wynder, J ... 1872*

X

Xanthakos, S A ... 3179
Xi, B ... 1740, 1741
Xi, S ... 2054*
Xi, Y ... 1647, 2070
Xia, H ... 2331, 2846, 2850
Xia, M ... 1095, 1811, 2462, 2586*, 2626, 2642, 3017, 3049
Xia, Y ... 1737, 3268
Xia, Z ... 1593, 1594
Xian, B ... 1810
Xiang, Y ... 3029, 3036, 3401
Xiao, G-H ... 1274
Xiao, H ... 2881
Xiao, Q ... 3529
Xiao, R ... 3212
Xiao, X ... 2588, 2889*
Xiao, Y ... 1589
Xiao, Y Q ... 1631
Xiao, Z ... 1405, 1919*
Xie, H Q ... 1189, 3423*
Xie, L ... 1435
Xie, W ... 1329*, 2843*, 3033, 3034
Xie, Y ... 1971
Xing, L ... 1619
Xiong, J ... 1444, 1907
Xiong, K-N ... 1444, 1907
Xiong, S ... 3075
Xu, C ... 1863*, 2883
Xu, G ... 2934*, 2955*
Xu, G-b ... 2665
Xu, H ... 1350, 1352*, 3423
Xu, J ... 1545, 2750, 2843
Xu, J Z ... 1072*
Xu, K ... 1479
Xu, L ... 1178, 1455, 1475, 1479*, 1483, 3473
Xu, M ... 3234
Xu, P ... 2061
Xu, S L ... 1189, 3423
Xu, T ... 1189, 2779, 3423, 3556*
Xu, W ... 2439
Xu, X ... 1261, 1890
Xu, Y ... 1251, 1267*, 2177
Xu, Y G ... 3526
Xu, Y-J ... 1678, 3171
Xu, Z ... 2600, 2601*
Xuan, J ... 2592, 3450*
Xue, K ... 1134*
Xue, K S ... 2272
Xue, P ... 1251, 3071

Y

Yabuki, M ... 1979
Yacov, G ... 2129
Yadav, J S ... 1296
Yadav, N ... 2245

The numerals following the author names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the presenter.

Yaeger, M J 1064, 3507
Yafune, A 2028
Yager, J 2701
Yager, J D 1084
Yakoub, Y 2249
Yakubi, H 1387
Yalcin, C O 2813
Yam, N 3514
Yamada, H 1824, 1868, 3267, 3456
Yamada, N 3182
Yamada, S 1827
Yamada, T 2156, 2476
Yamamoto, A 1567, 3514
Yamamoto, M 1251, 1567*, 2965
Yamamoto, S 1355, 1357*
Yamashita, H 1609*
Yamashita, K 3521*
Yamauchi, K 2919, 2920*, 2921
Yamazaki, H 1172, 3093
Yamazaki, R 1160
Yamazoe, Y 3456
Yan, B 3380*
Yan, C 1545
Yan, J 2758
Yan, T 1987
Yan, X 1831, 2051, 2969, 2973*
Yan, Z J 2674*
Yanagiba, Y 1406
Yanagisawa, H 3133
Yanamala, N 1291, 2803
Yanamala, N V 3496
Yang, B 1483
Yang, C 2163*, 2467, 2475, 2476, 2617, 2657, 2709
Yang, D 2944
Yang, E-J 2869
Yang, F-H 2383*
Yang, H 1597, 2301
Yang, I 2371
Yang, J 1483, 1809, 1860*, 1861, 2881, 2888, 2944, 3217, 3459*
Yang, J-H 1345
Yang, K 1120
Yang, M 2971*
Yang, P 1953
Yang, Q 3145*, 3146
Yang, X 1128, 1318, 1812, 1912*, 1916, 1943, 3438*, 3466*
Yang, Y 2218, 2662
Yang, Y-H 2383
Yao, J 1652, 2774*, 3064, 3072
Yao, P-L 2024*
Yao, Y 2322
Yaqub, L S 2913
Yasuda, M 3102
Yasuno, H 3522
Yasuoka, S 1160
Yates, E 2258, 3395
Yates, M D 1378
Yau, A 1682
Yauk, C 2088
Yde, C C 1124
Ye, H 1079*, 1865, 1870
Yeager, R 1605*
Yeakley, J 2655
Yedjou, C 1326, 1327, 1330*
Yeguvapalli, S 1076*
Yeh, A 2936*
Yeh, C-L 2354, 2355
Yemitan, O K 1466*
Yeo, H 1984
Yette, G 3281
Yeung, D 2103
Yeung, D T 2093, 2102, 2404
Yi, H 3074*
Yi, J 2817, 3238
Yi, K D 1694, 2917*
Yi, X 2745
Yi, Y-J 1546*
Yilmaz, S 1485
Yin, F 1128
Yin, J-J 2758
Yin, L 2896*, 3251, 3252
Yokoo, Y 2050
Yoo, D-H 2859
Yoo, J W 1400
Yoon, K S 2588, 2889
Yoon, M 1712*, 2014, 2188, 2975, 3464
Yoon, S 1423, 2724
Yoshida, E 1563, 1582
Yoshida, I 1393*
Yoshida, M 1960
Yoshida, S 3263*, 3264
Yoshida, T 2028, 3249
Yoshimoto, K 1567
Yoshioka, D 2784
Yoshitome, K 1354, 1355, 1356, 1357, 1358, 2784
Yoshizawa, Y 1239*
Yost, L 2166
You, D 2371*
You, H 3161
Young, J L 1539*
Young, R 1395
Young, R R 1411*
Young, S K 1958
Young, W 1638
Young, Y K 1554
Youngblood, B 3531
Yourick, J J 2771, 2789
Yourstone, S 2490
Yu, D 2746, 2747*
Yu, F 2479
Yu, H 1998, 3557
Yu, I J 1498, 2940*
Yu, L-R 3013*
Yu, M 1942
Yu, R 2086*, 2742
Yu, S 2178
Yu, X 1083, 1584, 2896, 3251, 3252*
Yu, Y 1797, 2888
Yuan, C 1519
Yuan, H 3051
Yue, M S 1185, 3542*
Yue, X 1329
Yue, Y 2881
Yuki, T 2732
Yun, C 1735
Yun, J 1735
Yunomae, K 1931

Z

Zaccaria, K 2699
Zaccaria, K J 2702
Zacharewski, T 1954
Zacharewski, T R 1100*, 1103, 1118
Zagorski, J 1346
Zagorski, J W 1344*, 1934
Zahedi, A 3550*
Zaikova, T 2792
Zaja, R 2945
Zaki, A R 1210
Zalups, R K 3120
Zaman, H M 3212*
Zamanian, M 2350
Zane, D 1374*
Zang, Q 1098*, 2202
Zani, B G 2442*
Zapata-Pérez, O 2953
Zarbl, H 2069, 3068
Zaroogian, G E 2933
Zasada, C 2338
Zastre, J 3413
Zati, D H 2855
Zauber, S E 2354, 2355
Zavala, J 1429, 1448*
Zawia, N 2385
Zayed, J 2693*
Zdawczyk, A 2446
Zeidan, R S 3157*
Zeidler-Erdely, P C 1289, 3485, 3488
Zeise, L 1717*, 2030, 3540
Zeiter, S 2442
Zeitlin, L 1626
Zekic, A 1382
Zelikoff, J 1846, 2247, 3248
Zelikoff, J T 1165, 1167, 1775*, 1847, 3381*, 3424
Zeller, A 1060
Zeller, K 2205
Zendejas, I 3169
Zeng, A 2366
Zeng, J 1831
Zeng, X 2312, 2555*
Zhan, L 1953
Zhang, C 3343
Zhang, D 3310*
Zhang, D D 2602
Zhang, F 2226
Zhang, G 2633*, 2750
Zhang, J 1976, 2282, 2284, 2352, 2590, 2673, 3187
Zhang, J G 3153
Zhang, L 1656*, 1961, 2060, 2301, 2417, 2420, 2426, 2435, 3164, 3430
Zhang, M 1597, 1688*, 2548
Zhang, N 1810
Zhang, P 1647
Zhang, Q 1251, 1688, 2214, 2804*, 3051*
Zhang, Q-Y 2292, 3147, 3458
Zhang, S 2403
Zhang, T 1213, 1663, 1946*, 3051
Zhang, W 1435
Zhang, W Q 3086
Zhang, X 1136, 1740*, 1741*, 1840, 1945, 2060, 2064, 2072, 2075, 2322, 3164, 3218*, 3338, 3478, 3479
Zhang, X-l 1590
Zhang, Y 1657, 1671, 1815*, 2769, 2774, 2804
Zhang, Z 2029, 2592
Zhao, B 1189, 2944, 3423
Zhao, C 1810, 1901, 1950*
Zhao, J 1597, 1811, 3051
Zhao, L 1084, 2338, 2906, 3292, 3408
Zhao, Q 1078, 1159
Zhao, Q J 2159, 2160, 2699, 2702
Zhao, R 1251
Zhao, Y 1128, 1656, 2188*, 2852
Zheng, C 3098
Zheng, C f 1486*
Zheng, J 2449
Zheng, N 2888, 2944
Zheng, W 1551, 1552, 1587*, 2132, 2363, 2366, 2830
Zheng, X 2811
Zheng, Y 2833
Zhong, L 1647
Zhong, M 1237, 3082*, 3088, 3513
Zhong, X 2013
Zhong, Y 1666*
Zhou, C 3224*
Zhou, G 1870, 2750, 3187
Zhou, H 3222*
Zhou, P 1600, 2112
Zhou, X 1275
Zhou, X D 3127
Zhou, Y 1453, 1998, 2446*, 2448
Zhou, Z 1860, 2963*
Zhu, B 2024
Zhu, H 2022, 2329, 2626, 2821*, 3509, 3510*
Zhu, J 1147, 2051*, 2969
Zhu, X 2029
Zhu, Y 1078*, 3361b*
Ziebel, J 2805
Ziemann, C 3484
Ziglari, T 1513*
Zimila, H 2921
Zimmerman, B 1671
Zimmermann, C 3060*, 3064
Zink, D 3075*
Zoeller, T R 2503b*
Zolkowska, D 1229
Zong, C 2060, 3164*
Zong, J 1483
Zorbas, M 1238
Zorrilla, L 3089
Zorzano, A 1806
Zota, A R 3141
Zou, C 3117
Zou, H 1688
Zu, K 2673*
Zucker, R M 2765
Zumbrun, E 1292
Zuñiga, G 2956
Zuo, R 3059, 3554
zur Nieden, N I 2311, 3286
Zurawski, V R 3061
Zurlinden, T 1369*, 3198
Zweier, J L 3399
Zychowski, K 1114, 1309*, 2261
Zylstra, G 3351

Notes

The numerals following each keyword refer to the relevant abstract number(s).

Abstract Keyword Index

Δ9-Tetrahydrocannabinol 1170
β-Methylamino-L-Alanine (BMAA) 2622
1,2,3-Trichloro-5-Nitrobenzene 3139
1,2-Dichloroethane 2033, 2232
1,3-Butadiene 3190
1,3-Dichloropropene ... 2042, 2674, 2900
1,4-Dinitrobenzene 3244
1,4-Naphthoquinone 2295, 2298
1-Bromopropane 1424
1-Hexanol 2154
2-(1,3,5-Dithiazinan-5-Yl) Ethanol 2662
2,3,7,8-Tetrachlorodibenzodioxin ... 3288
2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) 2055, 3268
2,2'-Thiobisethanol 2662
2,4,6-Trinitrotoluene 3451
2,4-D 1131
2-Deoxy-D-Glucose (2-DG) 1620, 1622
2Dg 1620
2-Hydroxymethacrylate 2445
2-Mcpd, 3-Mcpd 3028
2-Mercaptobenzothiazole 2138
2-Monochloro-1,3-Propanediol 3028
2-PAM 2848
3,3'-Diindolylmethane 2044
3,4-Dichloroaniline 2942
3,5-Dichloroaniline 3109
3D *In Vitro* Model 2186
3D Microtissues 2010, 2847
3D Models 3557
3-Hydroxy-3-Methylglutaryl Coa Reductase 1527
3-Methylcholanthrene 3158, 3160
3Rs 1630
4,4'-Methylenedianiline 3468
4-MCHM, PGPE 1441
4-Methylcyclohexanemethanol 2864, 2962
4-Tert-Octylphenol 2963
5-Aza-2'-Deoxycytidine 2061
5-Fu 3169
9,10-Phenanthraquinone, Endothelial Cell Dysfunction 2329
A-Carotenoids 2282
Abraxane 1202
Acamprosate 3085
Acceptable Daily Exposure 2682
Acetaldehyde 2144, 2216
Acetaminophen 1221, 1234, 1235, 1236, 1295, 1954, 1964, 1974, 1977, 1995, 1999, 2001, 2013, 2489, 3061, 3092, 3172, 3182, 3186, 3431, 3532
Acetaminophen Hepatotoxicity 1971
Acetaminophen, Mito-Tempo 1981
Acetic Acid 2694
Acetylcholinesterase 2095, 2886
Acetylcholinesterase Inhibitors 1212
Acetylcholinesterase, Antidote 2091
Acid Anhydride 2203
Acifluorfen 1960
Acrolein 1282, 1976, 2132, 2135, 2263
Acrylamide 1215, 1422
Acrylonitrile 2998
Activity Related Cytoskeletal (Arc) Associated Protein 1213
Acute Kidney Injury 2496
Acute Myeloid Leukemia (AML) 1663
Adaptive vs. Adverse 2212
Additive Manufacturing 2453
ADHD 1859
Adipogenesis 1555
ADME 2607
Adult Neurogenesis 1206, 2366
Adverse Outcome Pathway 2653, 2701, 3410
Aflatoxin 2653, 3012
Aflatoxin B1 ..... 1072, 1134, 1343, 1814, 1995, 3145
Aflatoxin M1 2271
Aflatoxins 2269, 2271, 2272, 3006
Aflatoxins, Fumonisins 2283
Agents 1223, 1271, 1292, 1446, 1512, 1549, 1682, 1932, 2046, 2093, 2110, 2111, 2123, 2124, 2479, 2672, 2806, 2829, 2894, 2913
Aging 1272, 1456, 1678, 1816, 1838, 1842, 2350, 2383, 2682, 2853, 2890, 3203
AhR 1185, 2428
AHR Repressor 2424
Air Liquid Interface 2815
Air Pollution 1167, 1299, 1450
Akt/S6K1 1475
Alcohol 1961, 1976, 2233
Alcohol Dehydrogenase 2430
Alcohol Ethoxylate Surfactants 1805
Aldehydes 1282
ALI 2788
Aliskiren 2720
Alkanolamine 2478
Alkenylbenzenes 1821
Alkylated Naphthalene AN-600 3441
Alkylating Agents 1062, 1389, 1600, 1915, 1989, 2097, 2099, 2110, 2111, 2112, 2113, 2115, 2123, 2124, 2126, 2128, 2152, 2483, 2496, 2854, 3115
All Trans Retinoic Acid 2599
Allergy 1851
Allyl Alcohol Carbon Tetrachloride . 3527
Alpha Synuclein 1595
Alpha-Cypermethrin, Pyrethroids 2902
Alpha-Synuclein, Parkinson's Disease 2342
Alternative Gene Splicing 2438
Alternatives Assessment 2692, 3410
Alternatives To Animal Testing 1101, 1105, 1121, 1150, 1153, 1154, 1171, 1224, 1399, 1415, 1429, 1506, 1522, 1615, 1630, 1636, 1788, 1789, 1801, 1803, 1810, 1814, 1820, 1821, 1992, 2014, 2030, 2058, 2171, 2172, 2176, 2177, 2178, 2180, 2181, 2182, 2184, 2186, 2187, 2188, 2190, 2190, 2193, 2194, 2195, 2196, 2198, 2199, 2201, 2202, 2203, 2204, 2205, 2208, 2223, 2287, 2446, 2451, 2452, 2456, 2462, 2468, 2470, 2471, 2474, 2550, 2551, 2606, 2623, 2625, 2626, 2628, 2629, 2632, 2646, 2651, 2652, 2656, 2710, 2737, 2772, 2783, 2815, 2822, 2860, 2863, 2891, 2989, 2990, 3017, 3057, 3058, 3060, 3063, 3064, 3066, 3070, 3072, 3074, 3077, 3110, 3111, 3113, 3119, 3151, 3192, 3222, 3266, 3279, 3280, 3388, 3515, 3516, 3540, 3547, 3550, 3557
Alzheimer's Disease 1842, 2397
Amatoxins 1963
Ames Test 2813
Aminophenol 2712
Amiodarone 3061, 3461
Amiodarone, Propylthiouracil 2006
Amitraz 2909
Amlodipine 2837
Amyloid 1587
Amyotrophic Lateral Sclerosis 2622
An Orphan Nuclear Receptor, NR2E3 1738
Androgen Receptro 2070
Anesthetic 1241
Aneuploidy 2738, 2773
Angiogenesis 1831
Aniline 3191
Animal Model 1237, 3087, 3088
Anniston 3419
Anthophyllite 1286
Anthrax 2217
Antibody Drug Conjugate 1619, 1930
Anticancer 1804
Anti-Factor Xa 2741
Antigen-Presenting 1347, 1361, 1362, 1368, 1928
Antimalaria 1662
Antioxidants 1133, 1197, 1210, 1345, 1456, 1484, 1485, 1556, 1557, 1568, 1579, 1581, 1662, 1817, 1923, 1975, 1981, 2047, 2212, 2296, 2314, 2318, 2333, 2395, 2406, 2451, 2587, 2595, 2600, 2602, 2802, 2885, 2891, 2913, 3024, 3225, 3245, 3341, 3430
Antituberculosis Drugs 1978
Antiviral 3549
Apoe -/- (Apolipoprotein E Knockout) 2859
Apoptosis 1102, 1315, 1318, 1320, 1321, 1322, 1323, 1324, 1326, 1328, 1329, 1330, 1354, 1357, 1427, 1483, 1656, 1831, 1951, 1967, 1968, 1976, 1993, 2044, 2048, 2078, 2333, 2334, 2396, 2598, 2761, 2843, 2853, 2854, 2880, 2973, 3229, 3245, 3290, 3545
Aqp4 2802
Aquatic Toxicology 1174, 1185, 1277, 1378, 1511, 1800, 1806, 2412, 2737, 2796, 2898, 2922, 2929, 2933, 2934, 2935, 2936, 2937, 2938, 2941, 2943, 2949, 2952, 2954, 2955, 3162, 3424, 3486
Archachatina Marginata 1463
Aristolochic Acid 1105
Aromatase 3393
Aromatic Amines 1820
Arrhythmia Detection 1646
Arrhythmias 2330
Arsenic 1249, 1250, 1252, 1253, 1254, 1256, 1259, 1260, 1261, 1262, 1263, 1264, 1265, 1266, 1267, 1350, 1352, 1478, 1599, 1691, 1834, 2052, 2149, 2168, 2667, 2749, 2982, 3339, 3340, 3341, 3343, 3344, 3345
Arsenic Biomethylation 2969
Arsenite 1275
Artificial Learning Models 2836
Artificial Turf 2670
Aryl Hydrocarbon Receptor 1185, 3285, 3287
Arylex, Rinskor 2458
As 1255
As3MT 2969, 3344
Asbestos 1281, 1312, 1354, 1356, 1357, 1690, 2047, 2139, 2317, 2695, 2696, 2867, 2868, 2974, 2979
Ascorbic Acid 2802
Aspirin 1328
Assessment 3193
Asthma 1295, 1851, 2694, 3212
Astrocytes 1322, 1580, 2337, 2352, 2377, 2381, 2802
Atherosclerosis 3344
Atopic Dermatitis 1468, 1517
Atosiban 3239
Atovastatin 3171
Atrazine 1792, 2894
Attention Deficit Hyperactivity Disorder 1637
AuNP 2792
Autism 1169
Autoimmune 1559, 1894, 1897, 1899, 1900, 1917, 2867, 2868, 2869, 2871, 2872, 2874, 2875, 2877, 2878, 3083
Autoimmunity 2870
Autonomic Imbalance 2260
Autophagy 1481, 3180
Azole 3280
Azoxymethane 1062
B Cell 1346, 1937
B Cell Development 1554
B16-F10 Melanoma Cells 2022
Baccharin 1663
Bacillus Subtilis, Probiotic 2268
Background 1684
Baicalin 3429
B(A)P-Metabolites 1213
Bark Beetles 2956
Bath Salts 2347
Batteries 2671
BCRP, Placenta 3235
BDE-47, BDE-99 ..... 3411, 3412
BDNF 2370, 2658
Behavior 1166, 1170, 1175, 1229, 1230, 1235, 1237, 1239, 1243, 1247, 1462, 1597, 1661, 1793, 1805, 1848, 1859, 2324, 2356, 2384, 2414, 2915, 2943, 2999, 3086, 3418
Behavioral 1169, 1795, 1808, 2105, 2122, 2374, 3000
Benchmark Dose 2165
Benicar, Crestor 1655
Benign Prostate Hyperplasia 1872
Benzene 2134, 2325
Benzene, Toluene, Ethylbenzene and Xylene (BTEX) 2922
Benzo[A]Pyrene 1213, 1946, 2040, 2953, 3283, 3284, 3392, 3462
Benzonatate 1391, 2019, 3260
Beryllium 1278
Beta-Naphthoflavone 3154
Betulinic Acid 1473
B-Glucan 1343
Bibliometrics 2995
Bifenthrin 2941
Bile Salt Export Pump (BSEP) 2731
Biliary Excretion 1966, 1985
Bioaccumulation 2925
Bioassy 1818
Bioavailability 1442, 1623, 2152, 2548, 2937, 2955, 3027, 3406, 3435, 3441, 3446, 3525
Biocompatibility 2452
Biodiesel 2803
Biodiesel Blend 2803
Bioenergetics 1798
Bioenergy Feedstock 1430
Biofuel 2459
Bioinformatics ... 1072, 1073, 1074, 1075, 1080, 1082, 1084, 1086, 1087, 1088, 1089, 1090, 1091, 1093, 1119, 2039, 2161, 2434, 2605, 2633, 2634, 2636, 2645, 2649, 2660, 2750, 2923, 2995, 3075, 3166, 3168, 3349, 3490, 3550
Biological Modeling 1144, 1204, 1437, 1440, 1504, 1526, 1670, 1872, 2039, 2061, 2144, 2185, 2215, 2217, 2218, 2219, 2222, 2225, 2233, 2234, 2240, 2349, 2441, 2452, 2493, 2607, 2608, 2633, 2646, 2665, 2681, 2716, 2937, 2944, 2979
Biologics 3440
Bioluminescence 2022
Biomagnification 2665
Biomarkers 1081, 1107, 1110, 1134, 1135, 1137, 1145, 1241, 1270, 1278, 1279, 1324, 1432, 1518, 1545, 1552, 1606, 1631, 1632, 1660, 1670, 1671, 1672, 1677, 1815, 1927, 1931, 1935, 2107, 2272, 2283, 2310, 2354, 2355, 2367, 2455, 2482, 2491, 2613, 2616, 2630, 2718, 2721, 2722, 2723, 2724, 2725, 2726, 2728, 2731, 2732, 2733, 2734, 2737, 2739, 2740, 2741, 2742, 2745, 2750, 2753, 2769, 2776, 2824, 2849, 2871, 3012, 3041, 3093, 3123, 3130, 3134, 3141, 3172, 3175, 3176, 3186, 3189, 3211, 3403, 3466, 3470, 3471, 3472, 3473, 3474, 3475, 3476, 3477, 3478, 3480, 3481, 3491, 3529, 3535, 3558
Biomonitoring 1129, 1130, 1135, 1136, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1260, 1646, 2022, 2851, 3141, 3211, 3351, 3556
Biomonitoring, Reverse Dosimetry 2229
Bioprocessing, Image Analysis, TEM, STEM 2540
Bioscavenger 2106
Biosimilar 1629
Biotech 3281

Biotech Products 1619, 1641, 1642, 1647, 1654, 1657, 2287, 2441, 2442, 2666, 3004, 3516, 3527, 3531, 3553
Biotransformation 1105, 1255, 1623, 1995, 1999, 2040, 2043, 2295, 2540, 2591, 2863, 3109, 3139, 3145, 3146, 3150, 3155, 3158, 3226, 3344, 3411, 3412, 3413, 3428, 3429, 3451, 3464, 3517, 3543
Biotransformation and Toxicokinetics 2038, 2197, 2219, 2231, 2727, 2905, 2945, 3273, 3428, 3436, 3438, 3439, 3443, 3444, 3453, 3460, 3461
Biphenyl 1881
Bismuth Oxide 2761
Bisphenol 2431, 2472, 2549
Bisphenol A (BPA) 1137, 1138, 1139, 1390, 1858, 1859, 1868, 1872, 1914, 2472, 3203, 3228, 3250
Bisphenol AF 2550
BLCO 3245
Bleomycin 1092, 2328
Blood Gas 1627
Blood Pressure 2264
Blood-Brain Barrier 2415, 2791
BM Niche Cells 1734
Bone 1552, 1555, 1735, 1883, 2318, 2442, 2736, 3048, 3151, 3288
Bone Marrow Progenitor Cells 3151
Bone Marrow, Monkey 1929
Boric Acid 3298
Botanical Supplements 2286
Botulinum and Tetanus Neurotoxins 3063
BPA 1138
BPA, BPAF, and BPS 1857
Brain 1167
Brain Blood Vessel 2802, 2809
Brain Uptake 3447
Breast Cancer 3342
Breast Cancer Incidence 2961
Brominated Flame Retardants 3390
Bromodichloromethane 1902, 2231
BTBD9 2365
Butyl Benzyl Phthalate 2705
Butyl Paraben 3292
Butyrylcholinesterase 1123, 2090, 2408
C. elegans 1813
C. elegans, Bisphenol A, Bisphenol S 1812
C. elegans, Mitochondrial Membrane Potential 1811
C-Abl Tyrosine Kinase 2373
Cadmium 1446, 1527, 1528, 1530, 1532, 1534, 1594, 1595, 1834, 2342, 2977, 3122, 3342, 3551
Cadmium Sulfate 3073
Caenorhabditis Elegans 1224, 1512, 1810, 1814, 3217
Caerulein 3473, 3474
Caffeic Acid 1402, 1416
Caffeic Acid Phenetyl Ester 1476
Caffeine 1845
Calcium Channels 3415
Cancer Therapy 1417
Cannabidiol, THC, Cannabidiol Oil 3282
Cannabinoid 3282
Cannabinoid Receptor 1342
Cannabinoids 1340, 1341
Cannabis 3403
Canthaxanthin 3005
Capsaicin 1469
Carbamate Pesticides 1348
Carbaryl 2394
Carbendazim 1893
Carbon Nanomaterial 2940
Carbon Monoxide 2170
Carbon Monoxide: Benzene, Tire-Derived Smoke, Scrap Tires 1685
Carbon Nanodots; Interference, Lactate Dehydrogenase Assay 2763
Carbon Nanotubes 3482, 3484, 3486, 3491, 3494, 3497, 3500, 3502
Carbonyl Protein, Superoxide Anion 2312
Carcinogenesis 1056, 1057, 1058, 1059, 1062, 1076, 1256, 1257, 1258, 1267, 1275, 1320, 1392, 1395, 1404, 1418, 1453, 1467, 1562, 1648, 1732, 1891, 1960, 2019, 2020, 2021, 2023, 2024, 2025, 2026, 2028, 2029, 2032, 2034, 2036, 2037, 2040, 2042, 2045, 2046, 2047, 2049, 2050, 2051, 2053, 2055, 2060, 2063, 2064, 2065, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2078, 2080, 2082, 2083, 2084, 2087, 2088, 2139, 2150, 2186, 2421, 2422, 2433, 2436, 2439, 2455, 2494, 2653, 2664, 2674, 2695, 2706, 2715, 2790, 3094, 3102, 3158, 3161, 3191, 3193, 3197, 3201, 3205, 3206, 3208, 3231, 3339, 3340
Cardiopulmonary 1067, 1068, 1070, 1114, 1297, 1309, 1397, 1622, 2135, 2243, 2260, 2261, 2262, 2263, 2330, 2838, 2844, 2848, 2855, 3100, 3103, 3137, 3402, 3507
Cardiotoxicity, miRNA 3475
Cardiovascular System 1114, 1185, 1266, 1325, 1329, 1496, 1520, 1553, 1622, 1625, 1646, 1665, 1666, 1740, 1741, 1742, 1743, 1744, 1829, 1830, 1836, 1844, 1845, 1846, 1879, 2130, 2134, 2143, 2177, 2248, 2261, 2265, 2331, 2449, 2600, 2601, 2647, 2676, 2817, 2818, 2819, 2820, 2822, 2823, 2824, 2826, 2827, 2828, 2831, 2833, 2835, 2836, 2837, 2840, 2841, 2842, 2843, 2845, 2846, 2847, 2849, 2850, 2856, 2857, 2858, 2859, 2973, 3045, 3100, 3101, 3108, 3163, 3209, 3238, 3277, 3399, 3417, 3466, 3531, 3542
Carrageenan 2589, 3009
Carvacrol 1975, 3022
Cataract 1605
Cattle 3021
CdO Nanoparticles 2758
Celastrol 1321
Cell Communication 1520, 2072, 2112, 2308, 2493, 2738, 3228, 3229
Cell Culture 1060, 1127, 1148, 1163, 1172, 1191, 1193, 1273, 1282, 1315, 1320, 1429, 1516, 1565, 1592, 1745, 1884, 1955, 1980, 1997, 2001, 2014, 2016, 2172, 2187, 2194, 2322, 2332, 2347, 2363, 2379, 2444, 2445, 2446, 2451, 2453, 2471, 2589, 2711, 2738, 2760, 2781, 2785, 2860, 3009, 3044, 3048, 3049, 3054, 3060, 3067, 3077, 3111, 3114, 3145, 3148, 3200, 3208, 3397, 3479, 3492, 3545, 3555, 3556
Cell Cycle 1838
Cell Lines, Transfected 1258, 1866, 2013, 2332, 2387, 2431, 2597, 2737, 3117, 3455
Cell Proliferation 1056, 1122, 1305, 1447, 1663, 1737, 1871, 1993, 2041, 2064, 2068, 2077, 2082, 2087, 2418, 2598, 3263, 3534, 3545, 3553
Cell Transformation 2081
Central Nervous System 2791
Cerebellum 1579
Cerebellum, Autism 3263
Cerium Dioxide 2541, 2542
Cerium Oxide 1496
Cerium Oxide Nanoparticles 2807
Cesarean 1851
Chasmanthera Dependens 1923
Checkpoint Inhibitors 1654
Chemical and Biological Weapons 1123, 1153, 1173, 1226, 1229, 1301, 1600, 1601, 1602, 1806, 2089, 2090, 2092, 2093, 2094, 2096, 2097, 2098, 2099, 2100, 2101, 2104, 2105, 2106, 2107, 2109, 2110, 2111, 2112, 2114, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2126, 2127, 2128, 2129, 2136, 2217, 2912, 3063, 3244, 3509, 3510
Chemical Allergy 1503, 1947, 1948, 2195, 2196, 2201, 2202, 2203, 2204, 2205, 2466, 2646, 2861, 2862, 2864, 2866, 2965, 2987, 3521
Chemical Categories 3081
Chemical Mixture 2193
Chemical Oxygen Demand 1276
Chemical Prioritization 2030, 2221
Chemical Toxicity Pre-Screening and Evaluation 1810
Chemical Warfare 1915
Chemokines 1335, 1733, 1924, 1936, 1974
Chemotaxis 1224
Chemotherapy 2048
Chemotypes 2709
Children's Health 1245, 1544, 1791, 2148, 2220, 2277, 2401, 2402, 2717, 2985, 3258, 3278
Chlordane 2159
Chlorine Gas 1301
Chloropicrin 2715
Chloropicrin and Hydrogen Fluoride 1601
Chloroquine 3221
Chlorpromazine 3461
Chlorpyrifos 1795, 2342, 2390, 2394, 2396, 2397, 2880, 2884, 3076
Cholesterol 1178, 1973
Cholinergic System 2405
Choroid Plexus 1221
CHPG 2370
Chromatin 3210, 3215, 3220
Chromium 1289, 1538, 1539, 3338, 3523
Chromium (VI) 2926
Chronic Liver Disease 3427
Chrysene 2058
Cigarette 3046
Cigarette Smoke 1106, 1337, 1837, 3045, 3221
Cigarette Smoke and Phthalates 3001
Cigarette Smoke Exposure 2290
Cinnamaldehyde 2480
Circadian 1230
Circadian Rhythm 3068
Cispaltin 1326
Cis-Permethrin 3448
Cisplatin 1331, 1401, 2496, 2721, 3115, 3126
Citizen Science 3351
Classification 1454
Clastogen 1410, 1422, 1426, 2782
Clastogenesis 1391
Clay Food Packaging 3020
Clinical Pathology 1632
Clinical Toxicology 1484, 1585, 1610, 1645, 1833, 1963, 2233, 2243, 2481, 2482, 2487, 2489, 2491, 2721, 2727, 2753, 2828, 2851, 2964, 3123, 3179, 3199, 3352, 3472, 3558
Clozapine 1333, 2828
CMP Slurries 2796
Coagulation 1276, 1951, 1954
Coal Fly Ash (CFA) 1285, 1430, 2244
Cobalt 1541, 1542
Cobalt Nanoparticles 2804
Cobinamide 2120
Cocaine 3174
Co-Culture System 2724
Co-Exposure 1592
Co-Exposure: Hepatic Toxicity, Cardiovascular Disease 1446
Collagen 2073, 2177
Colorectal Cancer 1328
Combretastatin 2032
Comet 3040
Comet Assay 2813, 3022
Comet in Nasal Tissue 1398
Complex Environmental Mixture 1423
Complex Mixture 1438
Complex Trait Genetics 2546
Computational Toxicology 1075, 1077, 1080, 1087, 1093, 1094, 1097, 1099, 1101, 1104, 1113, 1119, 1369, 1437, 1675, 1683, 1991, 2159, 2161, 2165, 2202, 2211, 2218, 2221, 2225, 2226, 2227, 2228, 2229, 2238, 2239, 2323, 2547, 2548, 2603, 2604, 2605, 2609, 2610, 2611, 2614, 2615, 2617, 2618, 2621, 2624, 2626, 2627, 2628, 2629, 2630, 2633, 2634, 2635, 2636, 2637, 2639, 2643, 2644, 2645, 2647, 2649, 2650, 2651, 2654, 2656, 2657, 2660, 2681, 2683, 2709, 3050, 3051, 3187, 3188, 3300
Conjugate 1653
Connective Tissue Growth Factor 2879
Constitutive Androstane Receptor 2434, 2435
Consumer Products 1679, 1681, 2671
Consumer Products Safety 3548
Continuous Infusion 1618
Control Banding 2693
Cookstoves 1302
Copper 1274, 1586, 2903
Copper Nanoparticles 2793
Copper Oxide 2786
Colorectal Cancer 2282
Corneal Endothelial Toxicity 1602
Cortical Pyramidal Neurons 1190
Corticosteroids, Calcinerurin Inhibitors 3083
Cortisol 3278
Cosmeceuticals 2778
Cosmetic Eye Irritation 2474
Cosmetics 2673
Coumarine-Type 1606
Covalent Crosslinker 1619
Cr (VI) 2075
Cramer Classification 2644
CRISPR/Cas9 1801
CRISPR/CAS-9, genome editing 3281
Crizotinib; Ceritinib 1318
Cross-Contaminant 2151
Crotonaldehyde 1282
Crude Oil 1439
Crude Oil and Dispersant Mixture 1815
Cumulative 2689
Cumulus Cells-Oocyte Complex 3228
Cupperoxide 2815
Curcumin 2212, 3071
Curcumin and Sulforaphane 1492
Curriculum Development 1379
Cutaneous/Skin Toxicity 1503, 1517, 1538, 1947, 2094, 2101, 2201, 2209, 2454, 2608, 2778, 2789, 2864, 2866, 2880, 3082, 3083, 3340, 3435, 3518, 3519, 3522, 3524, 3526
CXCR7, Endothelial Progenitor Cells 1831
Cyanide 2127, 2145
Cyanide Antidote 2127
Cyanobacterium 1924
Cyanotoxins 2147
Cyclic Ethyleneamine 1298
Cyclophosphamide 2854
Cyclosporin A 3461
Cyclosporine 3124
Cynomolgus Monkey 1633, 1639, 3186
Cynomolgus Monkey Infusion 1618
CYP19a1b 3283
CYP2F2 ..... 2036, 2038
CYP3A4 ..... 3049, 3146
Cyp3A-Null Mice, High Fat Diet 3156
Cyphenothrin 2882
Cysteine Adducts, Protein Adducts, Instability 1109
Cystic Fibrosis, HMGB1, Pseudomonas Aeruginosa 1912

Cytochrome P450 ..... 1111, 1419, 1492, 1860, 1873, 1875, 1902, 1920, 1955, 1998, 2002, 2031, 2036, 2038, 2060, 2292, 2304, 2427, 2438, 2489, 2946, 2956, 3036, 3043, 3076, 3131, 3139, 3141, 3142, 3143, 3146, 3147, 3148, 3149, 3151, 3152, 3153, 3154, 3155, 3156, 3157, 3158, 3159, 3160, 3163, 3164, 3165, 3179, 3184, 3207, 3241, 3246, 3270, 3353, 3411, 3412, 3434, 3450, 3451, 3456, 3458, 3459, 3463, 3465, 3479, 3543, 3555
Cytokine 1894
Cytokine Signaling 1183, 1212, 1253, 1340, 1344, 1352, 1554, 1664, 1902, 1922, 1943, 2290, 2293, 2322, 3009, 3039, 3508
Cytokines 1207, 1211, 1214, 1232, 1281, 1282, 1335, 1340, 1343, 1356, 1644, 1851, 1852, 1907, 1917, 1928, 1931, 1934, 1947, 1948, 1959, 2008, 2054, 2345, 2589, 2732, 2740, 2811, 2861, 2874, 2918, 3088, 3398, 3503, 3535
Cytotoxicity 1088, 1254, 1316, 1317, 1319, 1325, 1328, 1331, 1416, 1433, 1435, 1441, 1451, 1481, 1493, 1495, 1523, 1539, 1558, 1574, 1577, 1578, 1619, 1621, 1663, 1673, 1739, 1740, 1741, 1828, 1829, 1830, 1942, 1980, 2009, 2011, 2012, 2184, 2255, 2293, 2297, 2312, 2316, 2446, 2450, 2453, 2480, 2756, 2763, 2767, 2770, 2775, 2776, 2780, 2784, 2786, 2813, 2814, 2816, 2854, 2885, 2894, 2973, 3059, 3062, 3072, 3078, 3079, 3121, 3129, 3173, 3184, 3455, 3482, 3483, 3533, 3544, 3554
Daphnia Pulex 1816
Database 2605
DBP 2996
DDT 2665, 2955
DE-71 3177
Decamethylcyclopentasiloxane 2703
Decision Tree 2279, 2280
Decontamination 3509
Deltamethrin 2414, 2881, 3447
Deltamethrin, Adult Neurogenesis, Learning and Memory 2413
Deoxynivalenol 2276, 2873
Depleted Uranium 1561
Developmental 1368
Developmental or Reproductive Outcomes 3257
Developmental Toxicity; Post-Natal 1186, 1236, 1247, 1483, 1590, 1794, 1809, 1840, 1842, 1857, 1858, 1893, 2362, 2394, 2809, 2838, 2888, 2944, 3086, 3255, 3263, 3264, 3270, 3273, 3275, 3276
Developmental Toxicity; Pre-Natal 1082, 1175, 1177, 1182, 1196, 1359, 1589, 1590, 1839, 1843, 1845, 1846, 1850, 1856, 1857, 1868, 1880, 2177, 2472, 2749, 2771, 2817, 2911, 2983, 3194, 3203, 3232, 3258, 3265, 3268, 3271, 3272, 3273, 3290, 3345
Developmental/Teratology 1180, 1350, 1487, 1788, 1789, 1791, 1796, 1797, 1801, 1802, 1803, 1844, 1845, 1849, 2176, 2178, 2180, 2311, 2462, 2550, 2551, 2619, 2632, 2640, 2884, 2892, 2893, 2949, 2951, 3038, 3260, 3261, 3262, 3266, 3275, 3276, 3279, 3280, 3281, 3283, 3285, 3286, 3287, 3288, 3293, 3295, 3300
DFP 2405
DHA 1913
di-(2-Ethylhexyl) Phthalate 2057, 3297
Diabetes 1251, 1621, 2250, 2587, 2725, 2972
Diacetyl 1199, 1300, 2140
Diazinon: Sodium Arsenite, Toxicity, Catfish 2954
Dibenzo[Def,P]Chrysene 2079
Dibenzo[Def,P]Pyrene 3462
Dibromochloromethane 1407
Dibutyl Phthalate 2704
Dichloromethane 2039
Diclofenac 1998
Diclofenac Acyl Glucuronide 2316
Didecyldimethylammonium Chloride 2866
Dieldrin 2344, 2389, 2412
Diesel Engine Exhaust 2142
Diesel Exhaust 1169, 1431, 2251, 2976
Diesel Exhaust Particles 1207
Diet 1200, 3097
Dietary Fats 1080
Dietary Feeding 1893
Diethylene Glycol 3116
Diethylhexylphthalate 2046
Diethylstilbestrol 2546, 3213
Diindolylmethane 1917, 2080
Diisocyanates 2739
Diisopropyl Fluorophosphate 1214, 2406, 2409
DILI, Drug Property, Host Factor, Susceptibility 3183
Dimethylformamide 2190
Dinitrofluorobenzene 1503
Dioxin 1085, 1100, 1103, 1118, 1119, 1126, 1185, 1186, 1187, 1189, 1360, 1361, 1363, 1364, 1368, 1732, 1733, 1735, 1736, 1823, 1937, 2055, 2227, 2304, 2424, 2427, 2429, 2430, 2686, 2687, 2834, 2869, 2938, 3267, 3423, 3542
Diphenyl Ditelluride 1556
Discovery 3072
Disinfection By-Products 1306, 1319, 1407, 2231, 2866, 2996, 3135
Disposition 1678, 3441, 3444, 3452
Dissecting Aortic Aneurysm 2177
Diversity Outbred 1677, 2753
Diversity Outbred Mice 3089
DMBA 1874
DMSO 3049
DNA Adducts 1288, 1316, 1390, 1424, 2027, 2079, 2086, 2591
DNA Damage 1427
DNA Methylation 1263
DNA Repair 1062, 1275, 1387, 1390, 1392, 1397, 1402, 1404, 1405, 1414, 1417, 1425, 1977, 2494, 2926
DNA-Protein Crosslinks 1425
DOHaD 1792
Domoic Acid 3446
Dopamine Receptor Antagonist 1637
Dopamine, L-Dopa 2380
Dose Response 1298, 1300, 1326, 1328, 1424, 1536, 1542, 2039, 2108, 2133, 2138, 2149, 2154, 2155, 2160, 2165, 2166, 2170, 2596, 2649, 2688, 2695, 2700, 2713, 2717, 2730, 2751, 2768, 2976, 2992, 3008, 3014, 3106, 3250, 3514
Dosimetry 1508, 2210, 2719, 2765, 2994, 3437, 3485, 3533
Doxorubicin 2494, 2821, 2856, 3051, 3209, 3466
Drinking Water 2155, 2156, 2277, 2986, 2996
Drinking Water: Public Health 2152
Drosophila 1271
Drug Candidate Assessment 1675
Drug Induced Hepatotoxicity 2484
Drug Induced Liver Injury 2218
Drug Interaction 3432
Drug of Abuse 1339
Drug-Induced Liver Injury: Dili Classification; Database 3168
Drug-Induced Liver Toxicity 3450
Drugs 2750
Drugs of Abuse, Vmat2 1244
Dry Eye Disease 1610
Dyslipidemia 1249
Early B Cell Development 1360
Earthworm 1501
Ebola Virus 1626
Echocardiography 2263
E-Cigarette 2460
E-Cigarette Liquids 1338
E-Cigarette, Tobacco 2053, 3030, 3031
E-Cigarettes 1165
ECM 2177, 2211
Ecotoxicology 1501, 1519, 1522, 1696, 2031, 2344, 2635, 2757, 2919, 2920, 2922, 2923, 2926, 2927, 2928, 2929, 2942, 2948, 2951, 2953, 3298
Education 1376
Education Outreach Other ... 1272, 1372, 1373, 1374, 1375, 1377, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 2277, 2606, 2980, 2982, 3000, 3346, 3347, 3348, 3349, 3350, 3351, 3352
EGEHE 3444
EGME 3249
Eicosane 2666
Electroencephalography 1660
Electronic Cigarette: Vascular Endothelial Dysfunction, Smoking, Nicotine 3399
Electronic Cigarette 2480, 2638, 3032, 3048, 3397
Electronic Waste 1143
Electrophysiology 3264
Elk River Chemical Leak 2962
Embryo 1182, 2383, 2472, 3260, 3267, 3291, 3292, 3294, 3300
Embryonic Stem Cells 1184, 1737, 1741, 1822, 1823, 1829, 1832, 1835, 1839, 2407, 2480, 2640, 2771, 2852, 3073, 3074, 3247, 3409, 3466
Endocrine Disruptors 1084, 1089, 1137, 1141, 1161, 1188, 1188, 1268, 1390, 1437, 1792, 1827, 1850, 1855, 1856, 1857, 1858, 1859, 1864, 1867, 1868, 1870, 1892, 1897, 2057, 2069, 2214, 2545, 2619, 2631, 2704, 2705, 2718, 2744, 2904, 2933, 2941, 2947, 2963, 3213, 3230, 3236, 3250, 3283, 3292, 3298, 3343, 3390, 3393, 3394, 3422
Endocrine Estrogen 1862
Endocrine Toxicology 1089, 1860, 1861, 1862, 1868, 1882, 1884, 1887, 1888, 1889, 1891, 1896, 2174, 2439, 2651, 2916, 2943, 3084, 3089, 3251, 3389, 3393, 3394
Endocrine; Androgens 1098, 1188, 1870
Endocrine; Estrogens 1116, 1139, 1140, 1449, 1865, 1869, 1870, 1871, 1873, 1914, 2224, 2549, 2616, 2655, 3283, 3391, 3394, 3407, 3445
Endocrine; Other 1138, 1530, 1875, 1881, 2223, 2972, 3052, 3089
Endocrine; Receptor ... 1863, 1866, 1882, 1883, 2431, 3392
Endocrine; Thyroid 1147, 1162, 1195, 1885, 1886, 1887, 1888, 1889, 1890, 1892, 1893, 2911
Endogenous Activators 2299
Endometriosis 1914
Endometriosis; Epidemiology; Bias 2975
Endothelial Dysfunction 2134
Energetics 2937
Energy Drinks 2273
Engine Exhaust 1438
Engineered Nanomaterials 3110
Enniatins 2276
Enteric Nervous System 2375
ENU 1389
Environmental Exposures 3192, 3250
Environmental Fate 2932
Environmental Pollutant Mixtures 1429
Environmental Risk Assessment 2146
Environmental Toxicology 1074, 1079, 1090, 1128, 1265, 1285, 1319, 1352, 1376, 1379, 1403, 1418, 1435, 1441, 1475, 1479, 1501, 1505, 1519, 1562, 1570, 1582, 1593, 1683, 1685, 1691, 1695, 1696, 1807, 1816, 1872, 1905, 1908, 1932, 1949, 1962, 2075, 2214, 2229, 2241, 2244, 2254, 2266, 2290, 2300, 2323, 2324, 2346, 2386, 2456, 2461, 2622, 2663, 2729, 2744, 2779, 2791, 2898, 2928, 2930, 2939, 2946, 2950, 2954, 2956, 2971, 2984, 2992, 3021, 3068, 3131, 3196, 3202, 3210, 3211, 3271, 3284, 3289, 3343, 3404, 3424, 3537
Enzyme 1662
Epidemiology 1132, 1260, 1268, 1310, 1452, 1553, 2052, 2137, 2149, 2157, 2284, 2590, 2694, 2702, 2901, 2964, 2965, 2967, 2970, 2974, 2976, 2977, 2978, 2979, 2980, 2981, 2982, 2997
Epigenetics 1059, 1071, 1192, 1217, 1250, 1262, 1263, 1396, 1416, 1533, 1738, 1797, 1839, 1854, 1943, 2028, 2051, 2061, 2072, 2073, 2079, 2084, 2085, 2341, 2489, 2494, 2654, 2742, 2746, 2747, 2817, 2896, 2953, 2971, 3001, 3035, 3065, 3077, 3135, 3166, 3189, 3190, 3191, 3192, 3193, 3194, 3195, 3196, 3197, 3198, 3199, 3200, 3201, 3203, 3204, 3205, 3206, 3207, 3208, 3209, 3212, 3213, 3214, 3215, 3218, 3219, 3220, 3249, 3281, 3338, 3423, 3501
Epigenetics; Cell Transformation 2029
Epithelial to Mesenchymal Transition 2021, 3124
Epoxiconazole 2883
ER Stress 1481, 2767
Erlotinib 1972
Erlotinib Resistance 1664
Erythropoiesis 3295
E-Selectin; Nf-Kb, Are, H9C2 Cardiomyocytes 2821
Essential Element 1568
Esteroids 1141
Esters 2197
Estradiol 3388
Estradiol Benzoate 1873
Estrogen 2652
Estrogen Receptor 2439
ETBE 2700
Ethanol 1180, 1307, 1336, 2025, 2318, 3279
Ethanol Amines 3515
Ethinyl Estradiol 1871
Ethyl Acrylate 2063
Ethyl Methanesulfonate 1398
Ethyl Nitroso Urea 1411
Ethyl Tertiary-Butyl Ether 2031
Ethyl Vanillin 3519
Ethylbenzene 2038
Ethylene Dichloride 1179
Ethylene Glycol 3112
Ethylene Glycol Ethylhexyl Ether 3444
Ethynylestradiol 2933
Euphorbia Peplus 3257
Even-Carbon-Chain Alkanes 2666
E-Waste 3248
Excipient 1617
Excitotoxicity 1210, 1220, 1825, 2114
Exifone 2011
Exosomes 1136, 2351, 3175, 3181, 3339
Exposure Assessment 1129, 1131, 1136, 1139, 1140, 1145, 1249, 1430, 1497, 1508, 1526, 1545, 1548, 1567, 1584, 1596, 1601, 1639, 1642, 1654, 1679, 1681, 1682, 1684, 1686, 1689, 1690, 1692, 1693, 1694, 1695, 1698, 2146, 2156, 2197, 2226, 2234, 2267, 2270, 2272, 2276, 2278, 2283, 2461, 2466, 2663, 2671, 2672, 2673, 2677, 2682, 2693, 2694, 2739, 2741, 2792, 2799, 2917, 2962, 2966, 2998, 3024, 3035, 3037, 3046, 3106, 3155, 3206, 3400, 3403, 3405, 3419, 3440, 3495, 3525, 3539
Exposure Assessment Modeling 2281

Exposure, Environmental 1070, 1079, 1125, 1130, 1132, 1134, 1137, 1146, 1164, 1205, 1206, 1217, 1262, 1269, 1282, 1304, 1350, 1376, 1423, 1425, 1492, 1535, 1543, 1544, 1557, 1559, 1560, 1568, 1591, 1599, 1680, 1683, 1685, 1687, 1696, 1697, 1698, 1815, 1836, 1846, 1847, 1854, 1856, 1882, 1895, 1984, 2054, 2065, 2145, 2146, 2228, 2229, 2235, 2241, 2250, 2264, 2265, 2271, 2277, 2340, 2350, 2353, 2361, 2364, 2385, 2545, 2612, 2638, 2654, 2661, 2754, 2757, 2818, 2819, 2888, 2901, 2903, 2920, 2928, 2929, 2941, 2961, 2962, 2965, 2967, 2968, 2969, 2970, 2971, 2975, 2981, 2986, 2996, 3021, 3133, 3134, 3167, 3201, 3219, 3224, 3237, 3244, 3247, 3253, 3338, 3404, 3419, 3425, 3537, 3539
Extracellular Matrix 3388
Extrapolation Factor 2684
Extrapolation Modeling 2539
Eye Irritation 2193
Fc Gamma Receptor 2416
Fcgrii (Cd32) Antibody 1946
Fenton Metals 2254
Fertility 2735, 3221, 3223, 3228, 3238, 3239
Fetal Exposure 3278
Fgf21 2302
FGF21, Alcoholic Liver Disease, Lipolysis 1950
FGF21, IBD, Signal Transducer and Activator of Transcription-3 1901
Fiber Potency 2974
Fibrosis 1965
Fipronil 2882
Fischer 344 Rat 3139
Fish 2718, 2934, 2935
Flame Retardants 1796, 1797, 2185, 2669, 3247, 3405, 3408
Flavin Monooxygenase 2013, 2834
Flow Cytometry 1415, 1418, 1631
Fluconazole 3279
Fluoride 2277, 3130, 3133
Fluorescent Microscopy 1418
Food Allergy 2873
Food Carcinogen 1419
Food-Drug Interactions 3146
Food Proteins 3064
Food Safety/Nutrition 1066, 1264, 1265, 1388, 1480, 1494, 1514, 1814, 1874, 1963, 1992, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2288, 2457, 2467, 2473, 2543, 2589, 2590, 2591, 2592, 2617, 2668, 2781, 2968, 3004, 3005, 3006, 3007, 3008, 3010, 3011, 3012, 3013, 3015, 3016, 3019, 3023, 3024, 3025, 3026, 3027, 3028, 3045, 3078, 3125
Formaldehyde 1425, 1689, 2661, 2707, 2742, 3201
FOXO3 2311
Fracking Fluid 3248
Fracking Sand Dust 1284
Fragrance 3520
Fragrance Materials 2644
Free Radicals 1133, 3454
Fructose/Glucose 3013
Fulranumab 1641
Fusarium Mycotoxins 2276
GABA 2354
Gap Junctions 1444
Gasoline 1133
Gastrointestinal 1118, 1304, 1317, 1442, 1476, 1516, 1569, 1659, 1852, 1900, 1961, 1976, 2004, 2032, 2076, 2237, 2292, 2375, 2437, 2543, 2698, 3014, 3026, 3027, 3069, 3105, 3144, 3146, 3240, 3431
Gclm KO Mouse 1499
Gender 3448
Gene Co-Expression Modules 3140
Gene Editing 3281
Gene Expression 3078
Gene Expression Profiling 2724
Gene Expression/Regulation 1078, 1080, 1083, 1086, 1117, 1221, 1240, 1363, 1364, 1366, 1521, 1554, 1562, 1636, 1734, 1815, 1837, 1854, 1919, 1938, 1954, 1982, 2038, 2045, 2059, 2164, 2289, 2290, 2298, 2303, 2305, 2306, 2309, 2418, 2423, 2429, 2430, 2492, 2493, 2594, 2655, 2743, 2746, 2822, 2862, 2872, 2915, 3065, 3128, 3140, 3143, 3144, 3153, 3163, 3215, 3235, 3249, 3420, 3427, 3452, 3461, 3465, 3473, 3474, 3484, 3528
General Anesthetics 1840
Generalized Linear Model 1224
Genetic Polymorphisms 1278, 1311, 1364, 1569, 1593, 1800, 2052, 2067, 2481, 2748, 2750, 2751, 3234
Genetic Susceptibility 2052
Genetic Toxicology 1387, 1391, 1392, 1393, 1394, 1398, 1399, 1402, 1403, 1409, 1410, 1411, 1418, 1422, 1423, 1428, 1438, 1820, 1821, 2053, 2448, 2546, 2639, 2758, 2773, 2782, 3031, 3040, 3190, 3217
Genistein 1873, 1897, 2655
Genome 1087
Genome-Wide Analysis 2739
Genomics 1072, 1075, 1118, 1261, 2303, 2739, 2747, 3281, 3469
Genotoxicity 1060, 1108, 1274, 1330, 1389, 1395, 1396, 1397, 1400, 1401, 1402, 1406, 1407, 1408, 1410, 1412, 1413, 1414, 1415, 1416, 1420, 1421, 1422, 1425, 1426, 1438, 1480, 1539, 1549, 2138, 2248, 2294, 2592, 2593, 2639, 2695, 2697, 2761, 2768, 2772, 2782, 2804, 2808, 2813, 2914, 2971, 3020, 3022, 3066, 3077, 3157, 3190, 3197, 3213, 3217, 3487
Gfap 2802
Ginger 1210
Glia 1168, 1180, 1206, 1207, 1211, 1235, 1578, 1924, 2310, 2335, 2337, 2370, 2378, 2918, 3076
Global DNA Hypomethylation 3217
Glucose 1080
Glucuronidation 1138, 1998, 3456
Glutathione 1122, 1209, 1219, 1499, 1563, 2001, 2317, 2333, 2391, 2443, 2483, 3030, 3182
Glutathion-S-Transferases 2945
Glycemic Index 3010
Glycol Ethers 3515
Gold Nanoparticles 1504, 2766, 2794
Grapefruit Juice 3146
Graphene 2940, 3489
Graphical Analysis 2713
GRAS 2288
Green Fluorescent Protein 1939
Groundwater 1538
Guanidine Compounds 2157
Gut Microbiota 2883
Gymnemic Acid 1464
H1N1 2811
H2O2 2494
H9c2 Cells 2973
Hair Dyes 2466
Hair Relaxers 3394
Haloacetates 2312
Halobenzoquinone 1319
Halogenated Hydrocarbon 1179, 1367, 1424, 2060, 2232, 2330, 2454, 3164, 3411, 3412
Haloperidol 3085
Harm Reduction Tobacco Products 3286
Hazard Classification 2990
Hazardous Air Pollutants (HAPs) 1453
Heart Rate Recovery 3108
Hebei Spirit Oil Spill Accident 3217
Hematopoietic 1360, 1490, 1491, 1734, 1833, 1837, 1838
Hematopoietic Acute Radiation Syndrome (h-ARS) 3095
Hematotoxicity 1333, 1650, 1838, 2452, 2485, 2488, 2766, 3174, 3454, 3558
Hemodynamics 1674, 2831
Hemolysis 2447
Hemorrhage 1904
HepaRG 2013
Hepatic 1464, 1662, 1673, 1902, 1984, 1989, 1990, 2010, 2182, 2428, 2482, 3061, 3169, 3174, 3185, 3186, 3193, 3417, 3457, 3481, 3527, 3544
Hepatic Fibrosis 1996
Hepatic Stellate Cells 2493
Hepatitis B Surface Antigen 1927
Hepatobiliary 1668
Hepatocyte 3D Cell Culture 3148
Hepatocyte Spheroids 3557
Hepatocytes 1073, 1274, 1318, 1443, 1832, 1869, 1933, 1955, 1959, 1970, 1973, 1974, 1978, 1980, 1988, 1990, 1992, 1995, 1998, 2001, 2003, 2005, 2009, 2014, 2018, 2187, 2293, 2294, 2433, 2436, 2484, 2925, 3059, 3061, 3153, 3167, 3173, 3175, 3176, 3341, 3461, 3479, 3543, 3549, 3554
Hepatotoxicity 1967, 1968, 2016, 2728, 3049
Hepatotoxicity, Preclinical Safety, LC/MS/MS 3172
Herbicide 3449
Heterocyclic Amines 2372
Hexabromocyclododecane 1198
Hexahydro-1,3,5-Triazine-1,3,5-Triyl) Triethanol 2662
Hexanal 1282
Hexavalent Chromium 1536, 2697, 2698, 3225
HIF, Microbiota 1961
High Content 2596
High Content Analysis 1082
High Fat Diet 1858
High Throughput 2681
High Throughput Assay 2779
High Content Imaging 3118
High Content Screening 2711
High Throughput Screening 2161
Histone Acetylation 2085
Histone H3.1 2064
Histone Modification, RNA-seq, H3K18 2051
Histones 2072
Histopathology 1202, 1210, 1296, 1462, 1467, 1609, 1617, 1638, 1643, 1853, 1893, 1956, 1973, 2087, 2094, 2115, 2121, 2454, 2698, 2714, 2794, 2827, 3136
HL-60 Cells 1331
HMGB1 1909
Host-Associated Microbiome 1252
Household Insecticides 2987
hTERT(+/-) Knock Out Cell Lines 1512
HuH-7 Cells 3049
Human Adult Stem Cells 2178
Human B Cells 1363
Human Brain Tissue 2352
Human Cytokines 1560
Human Enterocytes 3146
Human Hepatocytes 3145
Human iPSC-Derived Neurons 1154, 1826
Human Mesenchymal Stem Cells (hMSCs) 2774
Human Neuroblastoma Cells 1218
Human Plasma 3145
Human-Induced Pluripotent Stem Cells 2339
Humanized Mice 3093
Huntington's Disease 2364, 2389
Huntington's Disease: Disease-Toxicant Interactions 2392
Huntington's Disease: Gene-Environment, Chlorpyrifos 2391
Hydrogen Cyanide 3106
Hydrogen Fluoride 2671
Hydrogen Peroxide 2210
Hydrogen Sulfide 1226, 2119, 2120
Hydroquinone 1427, 2325
Hydroxyethyl Methacrylate 2444
Hyperoxia, HMGB1 1916
Hyperoxic Lung Injury 3152
Hyperplasia 2087
Hypothalamic Pituitary Gonadal Axis 2735
Hypoxia-Inducible Factor 1α 2061
IATA, AOP 3003
IBM Watson 1093
Ibuprofen 3461
ICH M7 1400
ICV, Intrathecal Area 3099
Illicit Drugs 1157
Imaging 2856
Imatinib 1972
Imidazole 2190
Immune Suppression 1946
Immunocytokine 1644
Immunoglobulin Expression 1363
Immunotoxicity 1196, 1333, 1334, 1339, 1342, 1343, 1346, 1347, 1348, 1349, 1350, 1353, 1354, 1355, 1356, 1358, 1361, 1362, 1369, 1405, 1496, 1499, 1515, 1517, 1631, 1736, 1842, 1843, 1894, 1897, 1903, 1909, 1916, 1918, 1920, 1925, 1929, 1930, 1937, 2199, 2206, 2253, 2313, 2793, 2875, 3006, 3107, 3198, 3500
Immunotoxicology 1063, 1064, 1256, 1332, 1336, 1338, 1339, 1341, 1342, 1344, 1345, 1346, 1348, 1351, 1352, 1357, 1359, 1360, 1368, 1502, 1560, 1640, 1851, 1904, 1905, 1914, 1924, 1925, 1926, 1927, 1928, 1931, 1932, 1933, 1934, 1936, 1938, 1939, 1949, 2175, 2209, 2386, 2686, 2687, 2806, 2860, 2862, 2864, 2865, 2878, 3131, 3149, 3521, 3534, 3546, 3551
Impedance Pneumography 3103
Impressic Acid 1486, 1489
In Utero 1259
In Vitro 1974, 3109, 3215
In Vitro and Alternatives 1060, 1095, 1096, 1106, 1117, 1127, 1131, 1150, 1151, 1158, 1160, 1176, 1222, 1302, 1421, 1435, 1440, 1493, 1502, 1513, 1600, 1603, 1604, 1612, 1613, 1614, 1616, 1621, 1630, 1669, 1745, 1790, 1811, 1818, 1832, 1871, 1886, 1887, 1888, 1889, 1890, 1982, 1989, 1995, 1996, 1999, 2003, 2004, 2006, 2016, 2017, 2030, 2053, 2160, 2173, 2174, 2175, 2183, 2189, 2193, 2198, 2200, 2204, 2206, 2207, 2209, 2210, 2213, 2224, 2449, 2450, 2453, 2474, 2486, 2617, 2655, 2659, 2660, 2687, 2756, 2788, 2807, 2861, 2865, 2896, 2988, 3003, 3030, 3031, 3036, 3049, 3051, 3053, 3055, 3056, 3060, 3061, 3062, 3067, 3068, 3069, 3070, 3071, 3073, 3075, 3078, 3079, 3080, 3118, 3119, 3251, 3252, 3299, 3396, 3467, 3506, 3512, 3519, 3520, 3541, 3548, 3552, 3553, 3554
In Vitro Breast Cancer Model 3388
In Vitro Inhalation 3548
In Vivo Mutation 1411
Induced Pluripotent Stem Cells 1744, 1830
Induction 3049, 3153
Infection 1283, 1307, 1365, 1432, 1495, 1626, 1640, 1909, 1911, 1912, 1916, 1925, 1941, 2437, 2490, 2787, 3047, 3557
Infectivity 2811
Inflammasome 1226, 1523, 2119, 2373, 2880
Inflammation 1209, 1226, 1227, 1229, 1232, 1280, 1281, 1299, 1306, 1309, 1314, 1334, 1335, 1366, 1433, 1444, 1450, 1523, 1664, 1852, 1895, 1898, 1899, 1900, 1901, 1903, 1904, 1908, 1910, 1913, 1917, 1919, 1920, 1921, 1922, 1936, 1942, 1943, 1944, 1945, 1948, 1953, 2020, 2032, 2047, 2076, 2113, 2116, 2118, 2119, 2126, 2129, 2134, 2185, 2198, 2246, 2253, 2262, 2310, 2313, 2314, 2322, 2327, 2328, 2329, 2345, 2348, 2359, 2375, 2538, 2593, 2759, 2762, 2810, 2811, 2874, 2875, 2877, 3030, 3039, 3082, 3088, 3125, 3142, 3215, 3220, 3227
Influenza 2245, 2811
Infusion 2835, 2837, 3104
Inhalants 1065, 1337, 2135, 2330, 2855, 2859, 3034, 3395

Inhalation Toxicology 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1199, 1281, 1283, 1284, 1287, 1289, 1295, 1297, 1298, 1299, 1300, 1304, 1308, 1313, 1338, 1429, 1431, 1448, 1497, 1506, 1510, 1513, 1628, 1685, 1906, 1921, 2129, 2131, 2133, 2134, 2135, 2136, 2140, 2158, 2171, 2215, 2216, 2247, 2248, 2249, 2250, 2251, 2253, 2254, 2256, 2258, 2261, 2264, 2303, 2320, 2346, 2419, 2459, 2490, 2537, 2538, 2539, 2540, 2541, 2542, 2696, 2793, 2798, 2801, 2815, 2830, 2838, 2859, 2867, 2940, 2992, 2994, 3029, 3031, 3033, 3040, 3042, 3047, 3106, 3137, 3215, 3220, 3395, 3398, 3399, 3400, 3401, 3402, 3482, 3485, 3487, 3493, 3495, 3499, 3506, 3507, 3552
Inhalation 2671, 2791
Inhibition 3049
Innate Immunity 1851
Innovative Animal Model of Cancer 2022
Inorganic Arsenic 2620, 2701
Inorganic Chemicals 1276
Inorganic Mercury 1568
Insecticide 2411
Insecticide Resistance 2923
Insects 2930
Insulin 3086
Insulin Resistance 2972
Intercellular Adhesion Molecule-1 2821
Inter-Individual Variability 3433
Interleukin-1B 1232
Interleukin-17 2877
Interspecies Extrapolation 2994
Intestinal Microbiota 3429
Intestine 3009
Intranasal, Local Tissue 1643
Intrathecal Dose Administration 1633
Intrathecal Drug Delivery 1639
iPS Cells 1825, 1827
iPSC Derived Neuron 1172
Irinotecan 3452
Iron 1446
Ishikawa Cells, Human Endometrial Cells 1871
Isobutanol 2459
Isocyanates 2158
Isoflavones 1116, 1449
Isomer Toxicity 2712
Isoniazid 1918
Jatropha 2919, 2920, 2921
Jet Fuel 1205, 1434
JP-8 1204
Juvenile Dog 3107
Juvenile Toxicity 1142, 1170, 1173, 1637, 1879, 2942, 3243, 3269, 3274, 3275, 3276, 3277
Juvenile Zebrafish (*Danio rerio*) 2942
K–12 Outreach 1385
K–12 Stem Outreach 1372, 1384
Kairomones 2956
Kava Kava Extract 1609
Kidney 1271, 1463, 1531, 1532, 1540, 1571, 1653, 1671, 1672, 1678, 2085, 2599, 2611, 2720, 2722, 2723, 2910, 3028, 3053, 3097, 3109, 3110, 3111, 3112, 3113, 3115, 3116, 3117, 3118, 3119, 3120, 3121, 3122, 3123, 3124, 3125, 3128, 3130, 3132, 3133, 3134, 3135, 3137, 3138, 3139, 3140, 3189, 3297, 3449, 3470, 3471, 3529
Kinase 1666, 1674, 1740, 2048, 2335, 2826
Kinase Regulation 2070, 2306
Knockouts 1801, 1958, 1961, 2065, 2263, 2433, 2436, 3142, 3268, 3281
Knowledgebase 2238
Kola Acuminate 2059
Krebs Cycle Metabolites, Mesothelioma 1312
Kupffer Cells 1959
Label-Free Imaging 3545
Lapatinib 1972
Lambda-Cyhalothrin 2902
Larkspur 1461
Lead 1330, 1446, 1543, 1587, 1588, 1590, 1593, 2235, 2236, 2275, 2281, 2302, 2385, 2986, 3196, 3223
Lead Acetate 1842
Leptin 1530
Lewisite 2121
Life Cycle 3488
Ligand Selectivity 2299
Lindane 2389
Linear Mixed Models 2612
Lipid Rafts 1946
Lipids 1061, 1248, 1249, 1464, 1527, 1547, 1819, 1950, 1953, 1987, 2325, 2428, 2752, 2823, 2825, 2899, 3089, 3426
Lipopolysaccharide 1211, 1959, 2345
Liver 1058, 1073, 1083, 1091, 1110, 1111, 1113, 1128, 1318, 1431, 1527, 1540, 1571, 1572, 1668, 1669, 1670, 1677, 1736, 1820, 1821, 1863, 1877, 1918, 1940, 1949, 1950, 1951, 1952, 1953, 1954, 1955, 1957, 1958, 1959, 1960, 1961, 1962, 1964, 1965, 1966, 1969, 1970, 1971, 1972, 1975, 1976, 1977, 1979, 1981, 1982, 1983, 1985, 1986, 1988, 1991, 1993, 1994, 1997, 1999, 2000, 2002, 2003, 2004, 2006, 2007, 2008, 2011, 2013, 2015, 2016, 2017, 2025, 2028, 2037, 2043, 2050, 2084, 2173, 2211, 2213, 2286, 2291, 2302, 2319, 2422, 2432, 2435, 2455, 2491, 2611, 2703, 2726, 2727, 2731, 2733, 2754, 2883, 2935, 3005, 3049, 3054, 3091, 3092, 3145, 3150, 3166, 3167, 3168, 3169, 3170, 3171, 3172, 3177, 3178, 3179, 3180, 3181, 3182, 3184, 3189, 3241, 3460, 3463, 3467, 3468, 3472, 3476, 3480, 3481, 3549
Liver Enzymes 2794
Liver, Metabolic Disruption 3420
LLNA 3521
LMWH 2741
LNC RNA 2304
LOAEL, LD50 2683
Long Non Coding RNA 2026
Low Soluble Particles 1510
LPS 1490, 2348
Luciferase 2022
Lung Cancer 2149, 2976, 3212
Lung Cancer Mortality 2590
Lung Metastasis 2022
Lung Toxicity 1515
Lung; Pulmonary or Respiratory System 1064, 1067, 1106, 1125, 1253, 1279, 1280, 1283, 1284, 1285, 1288, 1294, 1295, 1296, 1300, 1302, 1303, 1307, 1308, 1310, 1311, 1313, 1314, 1323, 1444, 1521, 1529, 1558, 1561, 1627, 1687, 1904, 1908, 1911, 1913, 1915, 1945, 2036, 2140, 2188, 2244, 2245, 2249, 2259, 2317, 2326, 2327, 2328, 2480, 2490, 2602, 2638, 2696, 2715, 2759, 2770, 2783, 2800, 2810, 2868, 2879, 2940, 2978, 3029, 3033, 3035, 3037, 3041, 3147, 3397, 3400, 3401, 3425, 3458, 3488, 3489, 3492, 3493, 3494, 3495, 3496, 3502
Lycopene 2284, 2590
Lysosomes 1667
MAA 2745
Machine Learning 1098
Macrophage 1507, 1523, 1903, 1909, 1910, 1911, 1913, 1915, 1935, 1940, 2113, 2118, 2762, 2780, 3176, 3344, 3507
Magnesium 2457
Malathion ..... 2230 , 2905
Male Rats 3245
Mammary 2655
Mammary Gland 1857
Mammary Gland, Sholl Analysis, Sprague Dawley 3389
Mammary Gland, Uterus 1856
Mancozeb 2895, 2903, 2911
Maneb and Copper Chloride 2388
Manganese 2352, 2353, 2354, 2355, 2356, 2359, 2360, 2362, 2365, 2366, 2368, 2375, 2379, 2736, 2903
Manganese Toxicity 2361
Manganese, Huntington's Disease 2382
Maternal Transfer 3273
Mathematical Modeling 2646
MCHM 1445, 1693, 1793, 2864
MCHR1 1668
Mcl-1, Bcl-2 2334
MCLR 1479
MDMA, Ecstasy 1244
Mechanisms 1124, 1198, 1234, 1240, 1242, 1251, 1291, 1330, 1331, 1388, 1408, 1481, 1610, 1668, 1841, 1848, 1881, 1898, 1921, 1942, 1964, 1965, 1966, 1970, 1979, 2025, 2062, 2158, 2252, 2262, 2289, 2295, 2312, 2340, 2351, 2371, 2469, 2478, 2547, 2588, 2597, 2626, 2688, 2697, 2713, 2758, 2825, 2879, 3038, 3133, 3147, 3169, 3174, 3178, 3180, 3181, 3353, 3420, 3452, 3460, 3550
Mechlorethamine 2097
Medical Devices 2446, 2448, 2678, 2764
Medical Devices, Additive Manufacturing 2450
Medicinal Plant Toxicity 3463
MeHg 1565
MEHP 3294
Melamine 3471
Melamine and Cyanuric Acid 3470
Membrane Biology 2826
Membrane Integrity 1946
Membrane Transporter 2289
Mentoring 3346
Mercury 1349, 1567, 1584, 2285, 2937
Mesothelioma 1286
Metabolic 2135
Metabolic Activation .. 1105, 1250, 1407, 1866, 1869, 2321, 3044, 3090, 3157, 3185, 3546
Metabolic Disruptors 2733
Metabolic Syndrome 2587
Metabolism 1069, 1081, 1103, 1116, 1122, 1141, 1255, 1261, 1449, 1474, 1527, 1635, 1798, 1898, 1969, 2130, 2179, 2221, 2295, 2297, 2360, 2428, 2435, 2469, 2600, 2729, 2752, 2754, 2769, 2872, 2886, 2916, 2925, 2969, 3065, 3090, 3144, 3148, 3159, 3160, 3257, 3392, 3421, 3430, 3433, 3434, 3442, 3451, 3452, 3453, 3454, 3458, 3462, 3464, 3465, 3543
Metabolism, Mammary Gland 3391
Metabolome 1115, 3536
Metabolomics 1081, 1100, 1107, 1110, 1124, 1128, 1245, 1259, 1501, 1858, 2319, 2338, 2360, 2463, 2726, 2730, 2734, 2985, 3014, 3054, 3057, 3058, 3204, 3408, 3430, 3537
Metallothionein 1936
Metals 1125, 1132, 1143, 1249, 1250, 1251, 1252, 1253, 1255, 1256, 1257, 1258, 1259, 1260, 1261, 1262, 1264, 1265, 1266, 1267, 1268, 1269, 1270, 1271, 1272, 1273, 1274, 1276, 1277, 1278, 1314, 1350, 1351, 1353, 1401, 1413, 1446, 1478, 1513, 1528, 1530, 1531, 1532, 1533, 1534, 1535, 1536, 1537, 1538, 1539, 1540, 1541, 1542, 1543, 1545, 1546, 1547, 1548, 1549, 1550, 1553, 1554, 1555, 1558, 1559, 1560, 1561, 1563, 1564, 1565, 1566, 1567, 1568, 1569, 1571, 1572, 1573, 1575, 1576, 1584, 1586, 1587, 1588, 1589, 1595, 1597, 1598, 1599, 1691, 1809, 1834, 1835, 1879, 1883, 1936, 2073, 2075, 2081, 2137, 2237, 2267, 2278, 2281, 2285, 2302, 2315, 2342, 2358, 2359, 2363, 2366, 2368, 2379, 2382, 2388, 2450, 2538, 2620, 2668, 2735, 2736, 2749, 2760, 2765, 2766, 2767, 2788, 2795, 2798, 2799, 2801, 2890, 2950, 2977, 2981, 2984, 2984, 2986, 2993, 3002, 3032, 3033, 3120, 3122, 3127, 3132, 3134, 3196, 3204, 3223, 3225, 3248, 3252, 3290, 3338, 3339, 3342, 3343, 3345, 3416, 3551
Metalworking Fluid 2131
Metformin 2301, 2487
Methanol 1462
Methods/Mechanism 1117, 1455, 1661, 1667, 2012, 2168, 2183, 2400, 2469, 2487, 2653, 3068, 3085, 3092, 3128, 3282, 3530, 3551
Methylation 3209
Methylation Variance 3198
Methylene Chloride 2039
Methylenedioxypyrovalerone 2347
Methylglyoxal 3010
Methyllycaconitine 1461
Methylmercury 1563, 1566, 1569, 1570, 1573, 1574, 1576, 1577, 1578, 1579, 2289, 3027
Methylphenidate 3438
Microfluidic Chip 1810
Micro Rnas 3467, 3479
Microalgae 2929
Microarray 1078, 1092, 1403, 1451, 1541, 1609, 1664, 1802, 2607, 2728, 2809, 2952, 3050, 3143, 3216, 3236, 3340, 3408, 3469, 3484, 3485, 3535
Microbial Metabolites 3014
Microbiome 1115, 1270, 1569, 1791
Microbiome, Metagenomics Analysis 3018
Microbiota 1898, 1900, 2426
Microbes 1851
Microcystin 2147
Microcystin-LR 1475, 2935
Microelectrode Array (MEA) 1150
Microelectrode Array, Human Ips-Derived Neuronal Cells 1156
Microfluidics 3111, 3388
Microglia 1207, 2359
Micronuclei 2782
Micronucleus Assay 3022
Microrna 1432, 2350, 2489, 3138, 3190, 3207, 3211, 3466, 3469, 3470, 3471, 3472, 3473, 3474, 3477, 3480
Microrna, Regulatory T Cell 2862
Microsampling 1634, 3532
Microstructural Changes in Globus Pallidus 2367
Microsuspension Vehicle Emissions Expression Pro-Inflammatory Markers 1423
Microcystins 1224
Miniature Swine 3082, 3084, 3513
miR-122 ..... 3467, 3481
miRNA 2107, 3130, 3212, 3216, 3340, 3558
Mitochondria 1250, 1401, 1403, 1848, 1973, 1981, 2369, 2936, 3271, 3284, 3549
Mitochondrial 3092
Mitochondrial Dysfunction 1828
Mitochondrial Membrane Potential 1577
Mitochondrial Toxicity 1972, 1982, 3051
Mitochondrial Uncoupler 1798, 2332
Mitogen, Iron, Kupffer Cell, Hepatocyte, Endothelial Cell 3184
Mitomycin C 1316
Mitosis 1737
Mitotic Spindle 2773
Mixtures 1434, 1436, 1439, 1441, 1448, 1451, 1453, 1455, 1491, 1815, 2664, 2716, 2737, 2751
Mixtures, Generalized Concentration Addition 1437
MMP 1790
Mn 2367
Mode of Action 1881, 2031, 2068, 2138, 2478
Model 2275
Modified Risk Tobacco Products 1106
Molecular Dynamics 2398
Molecular Models 1369, 1867, 2338, 2381, 2398, 2652, 2956, 3159, 3467, 3538
Moniliformin 3008
Monkey 1931, 3005
Monoclonal AB 2740
Monomethylarsonous Acid 2051
Monosodium Glutamate 1210
Morphine 1239

Morpholino Knockdown 3038
Morus Alba 1458
Motor Dysfunction 1232
MPTP 2334
MRI 2409
MTBITC 1467, 2598
Multi-Electrode Array 1152, 1157, 1231
Multifunctional Acrylate 2152
Multiple Organ Culture 2188
Multiplexing 3553
Multiwalled Carbon Nanotubes 3487, 3493
Multi-Walled Carbon Nanotubes (MWCNT) 2773, 2782, 3485, 3488, 3498, 3505
Muscle Toxicity 1474, 1565, 2676
Mustard 1600
Mutagen 1409, 1423, 1426, 2063, 2932
Mutagenesis 1056, 1061, 1388, 1391, 1399, 1400, 1405, 1408, 1412, 1414, 1430, 1448, 2050, 2071, 2593
Mutation 1389, 1393
MWCNTs 3506
Mycotoxin 2272, 2831, 2991, 3008
Myocarditis 2828
N-(2-Aminoethyl) Ethanolamine 2177
N6AMT1 2969
N6-Formyllysine 2742
N7-Propyl Guanine 1424
Nafarelin 3239
NAFLD 3345
Nano Titanium Dioxide 2820
Nanocellulose 1291
Nano-Crystalline Cellulose 1291
Nanomaterials 2544, 2776
Nanoparticles 1167, 1168, 1174, 1296, 1313, 1497, 1498, 1500, 1502, 1507, 1510, 1511, 1518, 2171, 2249, 2539, 2540, 2541, 2542, 2543, 2755, 2756, 2759, 2761, 2764, 2770, 2772, 2774, 2777, 2778, 2780, 2783, 2784, 2786, 2787, 2788, 2789, 2790, 2791, 2793, 2794, 2796, 2798, 2799, 2800, 2801, 2802, 2804, 2809, 2811, 2814, 2815, 2818, 2819, 2820, 2830, 2858, 2940, 3006, 3110, 3482, 3483, 3486, 3487, 3488, 3490, 3491, 3492, 3493, 3494, 3496, 3497, 3498, 3499, 3500, 3501, 3502, 3503, 3504, 3505, 3508
Nanoporous Silica 1517
Nanosized Titanium Dioxide 2813
Nanotechnology 1291, 1496, 1499, 1501, 1503, 1504, 1505, 1506, 1507, 1508, 1509, 1512, 1514, 1515, 1516, 1517, 1519, 1520, 1521, 1522, 1524, 1525, 1526, 1688, 2449, 2537, 2719, 2720, 2757, 2758, 2760, 2762, 2763, 2764, 2765, 2766, 2767, 2768, 2769, 2771, 2773, 2774, 2775, 2779, 2781, 2784, 2785, 2792, 2795, 2796, 2797, 2805, 2806, 2808, 2810, 2812, 2816, 2820, 2858, 3238, 3281, 3486, 3489, 3494, 3496, 3497, 3498, 3500, 3502, 3505, 3506
Nanotitanium Dioxide 3238
Nanotoxicity 3495
Nanotoxicology 3496
Naphthalene 1288, 3147, 3458
NASH 1958, 3091
Nattokinase 3007
Natural Products 1085, 1088, 1297, 1315, 1327, 1433, 1447, 1456, 1457, 1458, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1467, 1468, 1469, 1470, 1472, 1473, 1476, 1477, 1478, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1557, 1662, 1663, 1818, 1853, 1968, 2023, 2035, 2044, 2056, 2148, 2271, 2276, 2473, 2485, 2587, 2589, 2592, 2827, 2919, 2921, 2968, 3009, 3017, 3018, 3019, 3021, 3056, 3080, 3126, 3257, 3261, 3429, 3511
Nematodes 2904
Neonicotinoid 2411, 2931
Nephrotoxicity 2724
Nerve Agent 2095, 2117
Nerve-On-A-Chip 1158
Nervous System 1069, 1149, 1151, 1165, 1184, 1199, 1205, 1230, 1233, 1242, 1244, 1461, 1579, 1645, 1652, 1661, 1808, 1950, 2100, 2315, 2366, 2376, 2387, 2408, 2415, 2416, 2464, 2707, 3085, 3108
Neurite Outgrowth 1222
Neuro2A 3056
Neuroblastoma 2024
Neurodegeneration 2341
Neurodegenerative Disease 1189, 2378, 2385, 2386
Neurodevelopment 1162
Neuroinflammation 1211, 1212, 1214
Neuromuscular Coordination 2913
Neurotoxicity 1224, 2346
Neurotoxicity; Developmental 1082, 1148, 1150, 1159, 1161, 1163, 1164, 1165, 1167, 1168, 1169, 1170, 1171, 1172, 1174, 1176, 1177, 1178, 1180, 1181, 1184, 1185, 1186, 1188, 1191, 1194, 1195, 1197, 1203, 1208, 1228, 1232, 1240, 1241, 1242, 1245, 1247, 1591, 1793, 1824, 1849, 1859, 1884, 1892, 1894, 2122, 2185, 2345, 2362, 2365, 2368, 2411, 2414, 2416, 2802, 2807, 3016, 3074, 3263, 3264, 3278, 3282, 3408, 3409
Neurotoxicity; Metals 1322, 1548, 1551, 1552, 1574, 1575, 1577, 1578, 1579, 1580, 1581, 1583, 1584, 1585, 1586, 1587, 1588, 1590, 1591, 1593, 1594, 1596, 1841, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2375, 2379, 2903
Neurotoxicity; Pesticides 1149, 1152, 1795, 2336, 2339, 2343, 2344, 2373, 2376, 2377, 2388, 2389, 2390, 2391, 2393, 2395, 2396, 2403, 2404, 2405, 2406, 2407, 2408, 2411, 2412, 2413, 2414, 2882, 2887, 2897, 2907, 3076
Neurotoxicology 1149, 1151, 1154, 1155, 1156, 1157, 1158, 1159, 1160, 1166, 1167, 1175, 1181, 1187, 1189, 1190, 1192, 1193, 1196, 1198, 1200, 1201, 1202, 1203, 1204, 1205, 1206, 1207, 1208, 1209, 1210, 1211, 1212, 1214, 1215, 1215, 1216, 1217, 1218, 1220, 1221, 1222, 1225, 1226, 1227, 1228, 1231, 1234, 1235, 1236, 1237, 1238, 1239, 1243, 1246, 1248, 1573, 1574, 1575, 1576, 1585, 1586, 1589, 1594, 1595, 1598, 1641, 1660, 1805, 1808, 1817, 1818, 1826, 1840, 1843, 2105, 2106, 2114, 2119, 2120, 2181, 2184, 2334, 2337, 2338, 2340, 2341, 2342, 2346, 2347, 2348, 2353, 2358, 2361, 2365, 2369, 2370, 2371, 2372, 2374, 2377, 2378, 2380, 2381, 2384, 2389, 2392, 2394, 2399, 2400, 2405, 2409, 2459, 2464, 2707, 2791, 2912, 3056, 3063, 3099, 3164, 3216, 3265, 3278, 3418, 3530
Neurotransmitter 1181, 1210, 1233, 1243, 2347, 2354, 2355, 2871
Neutrophil Infiltrations 1923
Neutrophils and Monocytes 1334
Nevirapine 1918
Next Generation Sequencing 2817
NHANES III 2282, 2284, 2590
Nickel 2073, 3210
Nicotine 1163, 2188, 3395, 3397
Nitric Oxide 3443
Nitrogen Dioxide 1070, 2141
Nitrogen Mustard 2099, 2113, 2115
Nitrosamines 2664
NK activity 1348
Non-Genotoxic . 1058, 1395, 2018, 2031, 2037, 2068, 2085, 2150, 2478, 3205
Nonhuman Primate 1840
Non-Mammalian Species 1187, 1419, 1588, 1792, 1793, 1796, 1797, 1799, 1804, 1813, 1817, 1819, 1994, 2357, 2424, 2931, 2933, 2935, 3038, 3136, 3162, 3542
Nonylphenol 2155
Norethindrone 2933
Noribogaine 1661
Nose 1288, 1298, 1643, 2144, 2216
Novelty Stress 1661
Nrf2 ..... 1344, 1934, 2594, 2597, 2598, 2602
Nrf2 signaling 2595
Nrf2, Autophagy 1482
NRU 2813
NSAID 1942, 1998, 2321
Nuclear Export 2296
Nuclear Receptors 1058
Nucleoside Analogs 1982, 2738
Nucleotide Excision Repair 3217
Nutraceutical 1447
OAT1 3117
Obesity 1061, 1464, 2588
Obesity, Diabetes 2889
Obesogens 2545
Obstetric Factors 1851
Occupational Exposure Limits 2142
Occupational Health 2966
Occupational Toxicology 1686
Occupationally Relevant Nano-Toxicity 3485
Ochratoxin A 2085
Ocular Irritation 2191
Ocular Toxicity 1601, 1602, 1603, 1604, 1605, 1606, 1607, 1608, 1609, 1610, 1611, 1612, 1613, 1614, 1615, 1616, 2115, 2116, 2121, 2189, 2190, 2192, 2486, 3142, 3207, 3269
OECD TG No. 453 2541
Oil Dispersants 2346
Olea Europaea 1457
Oleic Acid 2823
Olfactory 1598
Olfactory Bulb 2366
Olfactory Tubercle 1591
Olmesartan Medoxomil, Rosuvastatin Calcium 1655
Omeprazole 3145
Oncogene 2044, 2048, 2061, 2296
Ontogeny and Aging 1678
Optineurin 2376
Oral Carcinogenicity 1536
Organochlorine 2725
Organochlorine Pesticides 2910, 3227
Organometallics 1274
Organ-on-a-Chip 1148, 3541
Organophosphates 1123, 1212, 1465, 1850, 2090, 2091, 2092, 2093, 2095, 2100, 2101, 2102, 2103, 2104, 2106, 2107, 2109, 2117, 2279, 2390, 2392, 2393, 2397, 2398, 2400, 2401, 2402, 2403, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2456, 2848, 2887, 2899, 2905, 2912, 2913, 2918, 2947, 3093, 3265
Organosulfur 2891
Organotypic Cell Model 3408
Organotypic Culture, Fusion 2172
Organs-On-Chip 2187
Ortho-Phenylphenol 3435
Oseltamivir 2219
OSHA 2993
Osteoarthritis in Dogs 2473
Ototoxicity 1204, 1234, 1246
OVA 2471
Ovary 1327, 1855, 2070, 3221, 3222, 3224, 3226
Oxidative Injury 1102, 1200, 1219, 1266, 1289, 1303, 1326, 1337, 1428, 1466, 1798, 1809, 1967, 1972, 1974, 1981, 2027, 2097, 2125, 2212, 2243, 2246, 2265, 2311, 2313, 2314, 2315, 2318, 2319, 2320, 2323, 2324, 2325, 2327, 2328, 2329, 2343, 2383, 2386, 2391, 2443, 2444, 2445, 2483, 2595, 2599, 2745, 2803, 2804, 2821, 2870, 2910, 3015, 3067, 3071, 3126, 3152, 3154, 3254, 3286, 3295, 3398, 3399
Oxidative Potential 2256
Oxidative Stress 1210, 1518, 1923, 2317, 2360, 2594, 2808
Oxidative Stress, Hormesis 3341
Oxides Nanoparticle 3006
Oximes 2103
Ozone 1066, 1069, 1070, 1071, 1200, 2253, 2992, 2994, 3220
Ozone, Fish Oil, Olive Oil 1067
P47PHOX, TLR4 1852
p53 ..... 1316, 1413, 1977, 2024, 2099, 2853
PAH 1436, 2186, 2297, 2934
PAHs 1698, 2663, 3151
Pancreas 1638, 1650, 1875, 1877, 2972, 3291, 3292, 3294, 3414, 3473, 3474, 3477
Parabens 3273
Paracetamol 2484
Parallelogram Method 3406
Paraquat ... 2343, 2344, 2345, 2371, 2879
Parkinsonism 2355
Parkinson's, Synuclein 2380
Particle-Cell Interactions 2814
Particulate Matter 1216, 1217, 1846, 2143, 2246, 2265
Particulate Matter 2.5 2257
Particulates 1114, 1166, 1289, 1299, 1308, 1431, 1452, 1687, 1847, 1906, 1945, 2136, 2215, 2242, 2244, 2245, 2250, 2251, 2252, 2254, 2255, 2256, 2259, 2262, 2263, 2785, 2979, 3214
Pb 1544
PBDE 1197, 1408, 3141, 3236, 3409
PBIT 1223
PBPK Model (Rat) 2905
PBPK Modeling 1504
PCB126 3420
PCBs, Quinone 1109
PDE4 inhibitor 3042
Pediatric, NASH, VDRs 3179
Peginterferon Lambda-1A 3256
Peltophorum Africanum 1433
Perchloroethylene 2870
Percutaneous Absorption 2466, 3406, 3435, 3444, 3511, 3512, 3515
Percutaneous Penetration 3510
Perfluorinated Alkyl Substances 3258
Perfluorobutane Sulfonate 3439
Perfluorohexanoic Acid 1862
Perfluoronated Agents 1692, 1862, 1863, 1876, 1952, 1956, 1969, 2041, 2240, 2675, 2924, 2975, 3180, 3253, 3258, 3271, 3291, 3414, 3439
Perfluorooctanesulfonic Acid (PFOS) 1956, 3180
Peripheral Blood Mononuclear Cells 3534
Peripheral Neuropathy 1215
Permethrin 2414, 2658, 2889, 2897
Permitted Daily Exposure 2150
Persistent Organic Chemicals 1127, 1143, 1197, 1198, 1697, 1862, 1876, 2239, 2430, 2665, 2669, 2725, 2744, 2924, 3170, 3289, 3390, 3404, 3406, 3413, 3419, 3422, 3426, 3439
Persistent Organic Pollutants 1892
Pesticide Chemistry and Toxicology 2898
Pesticides 1057, 1130, 1131, 1135, 1144, 1147, 1171, 1173, 1323, 1348, 1465, 1694, 1792, 1806, 1877, 1891, 1960, 1968, 2049, 2102, 2117, 2192, 2341, 2342, 2344, 2371, 2373,2388, 2389, 2394, 2395, 2399, 2404, 2412, 2470, 2588, 2658, 2680, 2689, 2690, 2699, 2706, 2879, 2880, 2881, 2882, 2883, 2884, 2885, 2886, 2888, 2889, 2890, 2891, 2892, 2893, 2894, 2895, 2896, 2897, 2898, 2899, 2900, 2901, 2902, 2903, 2904, 2905, 2906, 2907, 2907, 2908, 2909, 2910, 2911, 2914, 2915, 2916, 2917, 2927, 2931, 2932, 2939, 2954, 2955, 3227, 3280, 3426, 3447, 3509, 3510, 3546
Petroleum 1686
Petroleum Hydrocarbons 1454
PFOA 1952, 2239, 3271
PFOS 1952, 3291
PFOS, PFOA 2675, 3253

Pharmaceuticals 1202, 1222, 1227, 1295, 1312, 1324, 1327, 1331, 1333, 1401, 1482, 1606, 1619, 1623, 1625, 1626, 1630, 1634, 1635, 1638, 1648, 1652, 1653, 1655, 1658, 1659, 1668, 1676, 1677, 1790, 1840, 1896, 1986, 2019, 2108, 2128, 2151, 2218, 2220, 2309, 2321, 2349, 2484, 2487, 2488, 2603, 2630, 2685, 2720, 2825, 2829, 2835, 2837, 2924, 2930, 2936, 2964, 2999, 3069, 3083, 3096, 3104, 3233, 3274, 3279, 3296, 3514
Pharmacogenomics and Drug Label 2750
Pharmacokinetics 1525, 1543, 1642, 2108, 2144, 2210, 2221, 2224, 2608, 2682, 3116, 3428, 3429, 3432, 3434, 3438, 3440, 3443, 3446, 3447, 3449, 3462, 3464, 3510, 3513, 3557
Phase Ii Metabolism 1885, 1979, 2598, 2945, 3445, 3456, 3517
Phenanthrene 2952
Phenobarbital 3145
Phenols 2279
Phenoxyethanol 2673
Phenylpyrazole Insecticide 2885
PhIP 2372
PHMB 3184
Phorate 2404
Phorbol 12-Myristate 13-Acetate (PMA) 2077
Phosphine 1323, 2130, 2303
Phosphoprotein, Signalling 1922
Photoageing 3218
Photosensitization 3055
Phototoxicity 2603
Phthalates 1142, 1182, 1305, 1345, 1412, 1440, 1855, 2046, 2057, 2071, 2704, 2705, 2985, 3224, 3255, 3293, 3294, 3297, 3405, 3540
Phthalocyanine 3525
Physiologically-Based Pharmacokinetics 1096, 1129, 1504, 1826, 2219, 2222, 2227, 2228, 2230, 2231, 2232, 2235, 2238, 2239, 2240, 2472, 2548, 2700, 2716, 3438
Picrotoxin 1220
Pig-A 1393, 1402, 1422
Pigment Decomposition 3525
Pioglitazone 1918
Piperazine Drugs of Abuse 1443
PKCδ 2373
Placenta 3236, 3237
Placenta, BCRP 3233
Plant Extracts 1493
Plasticizers 2924
Platelets 1496
Platycodin D 1460, 1488
Platycodon Grandiflorum 1470
Pneumocyte 1305
Poly Brominated Diphenyl Ethers ... 3177
Poly I:C, LPS 2175
Polyaromatic Hydrocarbon 1419
Polybrominated Diphenyl Ethers 3411, 3412
Polychlorinated Biphenyls 1109, 1191, 1192, 1193, 1194, 1195, 1421, 1592, 1962, 2612, 2730, 2834, 2938, 3170, 3289, 3415, 3415, 3416, 3417, 3418, 3419, 3420, 3425
Polycyclic Aromatic Hydrocarbons 1213, 1218, 1347, 1347, 1382, 1436, 1438, 1444, 1450, 1451, 1477, 1537, 1686, 1698, 1848, 1849, 1874, 1880, 1907, 1946, 2027, 2040, 2058, 2079, 2186, 2234, 2252, 2294, 2297, 2308, 2320, 2419, 2934, 2949, 2951, 2952, 2953, 2961, 2980, 3043, 3150, 3160, 3161, 3162, 3165, 3221, 3245, 3246, 3285, 3287, 3288, 3392, 3424, 3462
Polyethylene Glycol 1653
Polymorphisms 2969
Polymyxin 3119
Polyploidization 1421
Ponatinib 1650
POPs 2631
PPAR 1987
PP2A 1475
Pravastatin 3233
Precision-Cut Liver Slices 1997
Predictive Toxicology 1083, 1091, 1099, 1113, 1120, 1158, 1194, 1225, 1443, 1454, 1614, 1615, 1636, 1653, 1669, 1674, 1675, 1676, 1742, 1803, 1828, 1865, 1881, 2006, 2017, 2030, 2049, 2163, 2172, 2176, 2180, 2188, 2191, 2194, 2196, 2203, 2222, 2238, 2280, 2467, 2475, 2476, 2486, 2495, 2549, 2603, 2604, 2610, 2615, 2618, 2619, 2623, 2624, 2625, 2627, 2628, 2629, 2637, 2642, 2647, 2650, 2653, 2657, 2677, 2849, 2988, 3059, 3073, 3075, 3080, 3081, 3113, 3118, 3121, 3129, 3168, 3173, 3188, 3264, 3436, 3497, 3539, 3540, 3541, 3549
Pregnancy 3240
Prenatal Exposure 1135, 2793, 2963
Preservatives 2474
Preterm Birth 3227
Proallium, Active Food Packaging .. 3023
Prochloraz 2068
Proliferation 2018
Pro-Oxidants 3295
Propargite 1395
Propofol 2964
Propolis 1210, 1494
Proposition 65 ..... 2661, 2667
Propylene Glycol 2638
Prostate 1256
Prostate Cancer 2059, 2284, 2333
Protein Allergy 2471, 2732
Protein Corona 2760, 2766
Protein Phosphatase 1224
Protein Structure 2398, 3538
Proteome 1109
Proteomics 1092, 1109, 1123, 1417, 1500, 1518, 1524, 1657, 1790, 2000, 2211, 2308, 2380, 2445, 2728, 2734, 2887, 3013, 3041, 3172, 3490
Proteomics, Cardiac 2412
Proteomics Phenomics 1501
Proteostasis 1199
Psuedomonas Putida Biosensor 2757
PTSD 1943
Pulmonary Fibrosis 1092
Punicic, Jacaric, Lithocholic Acid 1481
PXR 2437
Pyrethroid Insecticides 1135
Pyrethroids 2415, 2987
Pyridostigmine Bromide 2104, 3108
Pyridoxine 3016
Pyrrolizidine Alkaloids 1992
Pyrrolizidine Alkaloids (PAs), Hepatic Nonparenchymal Cells 3185
Pyruvate 1566
QSAR 1096, 1097, 1098, 1099, 1400, 1445, 1680, 1865, 1886, 1991, 2159, 2161, 2191, 2226, 2615, 2618, 2621, 2625, 2627, 2648, 2650, 2652, 2659, 2708, 3415, 3511
Quantum Dots 1499
Quaternary Ammonium Compounds 1178
Quercetin 3245
Rabbit 2914
Radiation, Anthracycline 1397
Radiotoxicology 2688
Radon, Cigarette Smoking 3206
rasH2 3102
rasH2 mice 2033
RD50 2621
Reactive Intermediate 2011, 2312, 2316, 2321, 2380, 3433, 3454
Reactive Oxygen Species 1481
Reactive Oxygen Species; Isomer Effects 1319
Reactive Sulfur Species 2298
Reactivity 2820
Read-Across 2709, 2988
Receptor 1336, 1987, 2024, 2041, 2416, 2418, 2421, 2434, 2435, 2481, 2492, 3086, 3415, 3422
Receptor; Aryl Hydrocarbon 1176, 1189, 1190, 1359, 1365, 1366, 1367, 1450, 1732, 1733, 1734, 1737, 1738, 1823, 1837, 1937, 2058, 2076, 2299, 2304, 2305, 2309, 2417, 2419, 2420, 2422, 2423, 2424, 2425, 2426, 2427, 2429, 2597, 3177, 3200, 3353, 3423, 3427, 3459, 3538
Receptor; G-Protein Coupled 1342
Receptor; Nuclear Hormone 1077, 1799, 2020, 2433, 2436, 2437, 2438, 2439, 3240, 3457, 3508
Recombinant Inbred Mice, Sex Differences, QTL 2336
Reconstructed Human Airway 2175
Reconstructed Human Epidermis (RHE): Skin Irritation 3547
Recovery 2340
Recycling 1143
Red Mercuric Sulfide 1571
Redox Proteomics 1125
Regulatory, Dose-Response 2719
Regulatory/Policy 1510, 1536, 1538, 1616, 1689, 1692, 2133, 2148, 2156, 2157, 2162, 2166, 2275, 2288, 2448, 2460, 2468, 2470, 2632, 2641, 2643, 2677, 2683, 2685, 2689, 2692, 2717, 2974, 2986, 2987, 2988, 2989, 2991, 2993, 2994, 2995, 2996, 2997, 2998, 3000, 3001, 3002, 3003, 3034, 3058
Renal 1650, 2496, 2700, 2721, 3114, 3117, 3126, 3127, 3129, 3136
Repeated-Dose Toxicity 2650
Reporter Assay 2058
Reproductive and Developmental Toxicology 1121, 1177, 1179, 1487, 1624, 1789, 1796, 1814, 1816, 1844, 1847, 1851, 1853, 2270, 2411, 2547, 2550, 2648, 2735, 2755, 2909, 2942, 2963, 2977, 3192, 3194, 3225, 3231, 3232, 3233, 3236, 3239, 3247, 3248, 3253, 3255, 3256, 3259, 3260, 3261, 3262, 3266, 3282, 3284, 3299, 3390, 3449
Reproductive System 1812, 1874, 2023, 2147, 2284, 2635, 2914, 3239, 3242, 3250, 3257
Reproductive Toxicity 3298
Reproductive Tract; Female . 1432, 1914, 2546, 2549, 3070, 3199, 3228, 3541
Reproductive Tract; Male 1104, 1546, 2055, 2174, 2704, 2705, 2803, 3244, 3245, 3246, 3251, 3252, 3254, 3255, 3442
Respiratory 1063, 1068, 1284, 1309, 1452, 1628, 1665, 1907, 1978, 2131, 2141, 2245, 2255, 2257, 2259, 3042, 3047
Respiratory Sensitization 2694, 3214, 3503
Respiratory Toxicology 1063, 1065, 1279, 1280, 1290, 1291, 1292, 1293, 1301, 1303, 1307, 1311, 1338, 1362, 1411, 1627, 1628, 1684, 1906, 2125, 2130, 2132, 2139, 2142, 2175, 2246, 2258, 2542, 2659, 2710, 2787, 2800, 2823, 2900, 3039, 3042, 3101, 3154, 3161, 3395, 3396, 3489, 3498, 3505, 3552
Resveratrol 1292, 2833, 3225
Retina 1608
Retinoic Acid 3300
Retinol Signaling 2462
Retractions 3000
Retrorsine 3185
Reversibility 2354
Rheumatoid Arthritis 1917
Rhus Trilobata 1493
Rifampicin 3153
Rifampin 3145
Risk Assessment 1087, 1203, 1264, 1277, 1286, 1310, 1398, 1436, 1441, 1453, 1454, 1596, 1603, 1640, 1674, 1679, 1682, 1684, 1690, 1691, 1693, 1694, 1695, 1743, 1800, 1825, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2145, 2147, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2159, 2160, 2162, 2163, 2164, 2165, 2167, 2168, 2169, 2170, 2183, 2195, 2197, 2214, 2227, 2232, 2233, 2236, 2270, 2278, 2281, 2287, 2288, 2458, 2468, 2475, 2476, 2631, 2634, 2636, 2641, 2643, 2648, 2657, 2661, 2662, 2663, 2664, 2665, 2666, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2678, 2679, 2680, 2681, 2683, 2684, 2689, 2690, 2691, 2692, 2693, 2696, 2697, 2698, 2699, 2701, 2702, 2703, 2704, 2705, 2706, 2707, 2708, 2709, 2710, 2712, 2713, 2715, 2716, 2718, 2719, 2742, 2857, 2884, 2900, 2944, 2962, 2963, 2974, 2984, 2997, 2998, 3002, 3015, 3034, 3051, 3071, 3081, 3093, 3095, 3183, 3405, 3410, 3523, 3528, 3533
Risk Perception 2901
Risperidone 3086
RLS 2365
RNA-seq 1072, 3037, 3144
Rodent Cage Environment 1649
Rofecoxib 2032
Romania 2276
ROS 1357
ROS, NF-kB 2329
Rosin 1442
Rostral Migratory Stream 2366
Rotenone 2340, 2373, 2376, 2377
RT-PCR 1078, 1273, 1302, 1356, 1592, 1860, 2059, 2109, 2492, 3016, 3130, 3132, 3227
Rutaecarpine 1472
RyR2 3422
Safety Assessments 1867
Safety Evaluation 1179, 1396, 1398, 1411, 1434, 1457, 1458, 1459, 1471, 1604, 1612, 1613, 1617, 1618, 1620, 1621, 1624, 1629, 1637, 1641, 1645, 1647, 1648, 1649, 1652, 1655, 1665, 1671, 1672, 1675, 1743, 1745, 1804, 1927, 2008, 2146, 2163, 2189, 2199, 2206, 2207, 2258, 2273, 2274, 2279, 2280, 2440, 2441, 2442, 2447, 2448, 2459, 2461, 2463, 2464, 2465, 2467, 2474, 2475, 2476, 2488, 2539, 2544, 2587, 2614, 2638, 2662, 2666, 2667, 2669, 2673, 2679, 2685, 2703, 2709, 2723, 2832, 2836, 2865, 2987, 2989, 2991, 3004, 3005, 3011, 3019, 3025, 3057, 3079, 3087, 3096, 3098, 3262, 3274, 3471, 3519, 3520, 3528, 3547
Safety Pharmacology 1112, 1172, 1241, 1466, 1494, 1608, 1622, 1625, 1627, 1634, 1646, 1659, 1660, 1673, 1742, 1744, 1826, 1830, 1988, 2007, 2009, 2220, 2477, 2778, 2832, 2835, 2836, 2837, 2839, 2840, 2842, 2857, 2878, 2999, 3100, 3101, 3514, 3531, 3544
Salicylates 1909
Saliva 1130
Salvia Plebeia 1468
Schizophrenia 1652
Seahorse Bioscience 1798
SEB 1292
Second Hand Smoke 3212
Selected Reproductive Hormones 2735
Selected Trace Metals 2735
Selenium 3290
Seleno-L-Methionine 1816
Senescence 1550
Sensitivity 3255
Sensory Neurons 1224
Sepsis, Heart Failure, Serca2A 2833
Sewage Water 1137
Sickle Cell 2968

Signal Transduction 1219, 1251, 1336, 1349, 1482, 1563, 1739, 1827, 1919, 1922, 1940, 2000, 2078, 2081, 2291, 2296, 2298, 2299, 2306, 2308, 2334, 2351, 2396, 2444, 2855, 3094, 3165, 3268
Silica 1284, 1313, 1921, 2759, 2874
Silver 1174, 1511, 2765, 2771, 2788, 2799
Silver Nanoparticles 2181, 2757, 2767, 2789, 2806, 2814
Single-Walled Carbon Nanotubes 2811, 3495
SiO2 2540
SiO2, SLGO 1512
SIRC Cell Line 2193
SiRNA 2289, 2387, 2595, 2879
Sirt1, Cholestasis 1966
Skin 1267, 2628, 2629
Skin Lesions, Skin Cancer 2052
Skin Penetration 2789
Skin Sensitization 2198, 2208, 3523
Skin Sensitization Potency 2863
Skin Sensitization, Allergy 3520
Skin Wound Repair 2112
Skin, Microvesication, Silibinin 2126
SM 2112
Small for Gestational Age 2983
Smoke 1837, 3041
Smokeless Tobacco 3066
Smoking, Asbestos 1310
SNPs 1285, 2052, 2481, 2749, 3177, 3234
Socioeconomic Status (SES) 2984
Sodium Fluoride 2594, 2973
Sodium Fluoride 2973
Sodium Tungstate Dihydrate 1353
Soy Isoflavones 1140
Soy Protein Isolate 1874
Sparstolonin B 1949
Spatial Discrimination Reversal Task 1213
Sperm 1546, 2069, 2812, 2931, 3248
Spheroids 1995
Squamous Metaplasia 1298
SR141716bA 1898
Stable Isotope Dilution, Drug Release Method 1525
Stably Expressed Genes 1111
Steatohepatitis 1953, 3170
Steatosis 2005
STE 2193
STEM 1380
Stem Cells 1267, 1351, 1834, 2374, 2764, 3076, 3339
STEM Education 3347
Steroid Hormones 1255
Steroidogenesis 2637
Steroidogenic Enzymes, Brain, Clarias Gariepinus 3297
Striga Hermonthica, Rats, Toxicity 1462
Styrene 2036
Substituted Phenoxyalkyl Pyridinium Oxime 2109
Subventricular Zone 1551, 2366
Sulfation 3455, 3457
Sulfolane 3453
Sulfonylurea 3449
Sulfur Dioxide 2602
Sulfur Mustard 1602, 2116, 2125
Sunitinib 1972
Super-Resolution Microscopy 2332
Supravital 1418
Surface Plasmon Resonance 3551
Surfactants 2474
Susceptibility Exposure Paradigm 1213
Swine 3106
Sympathetic 2855
Synaptic Neurotransmission 3063
Synergy, Lung Cancer, Threshold 1310
Synthetic Cathinones 1967
Synthetic Ecm 3388
Synthetic Pyrethroids 2914
Synucleinopathy, Parkinson's 1599
System Biology 2631
Systematic Review 2166, 2632
Systems and Integrative Toxicology 1069, 1084, 1086, 1100, 1102, 1103, 1106, 1107, 1115, 1234, 1439, 1572, 1954, 2325, 2631, 2640, 2641, 2712, 2939, 3003, 3013, 3036, 3091, 3171, 3402
Systems Biology 1066, 1077, 1094, 1101, 1104, 1108, 1116, 1120, 1121, 1124, 1252, 1419, 2081, 2212, 2213, 2440, 2495, 2832, 2985, 3037, 3043, 3171, 3349, 3401, 3527
T Cell 1356
Tamiflu 2219
Tanezumab 1238
Taurine 1181
Taurine, Chlorpyrifos 2913
TBB, TBPH 3406
TBBPA 2045, 3407, 3445
TBEP 2947
tBHQ 1344, 1346
TCDD 1360, 1366, 1937, 2227, 2422, 2424, 3427, 3542
TCPOBOP 2435
TDAR 1926
TDCPP 1797, 1813
TDI 2862
Tear Film 1610
Tecovirimat 2108
Telemetry 2839
Tellurium Tetrachloride 1556
Telomeres 1272, 1512
Tempol 3430
Terminalia Chebula Extract 2473
Terpineol 3442
Tert-Butanol 2700
Testis 1873, 2803, 3249, 3254, 3298
Tetrabromobisphenol A 1856, 2045
Tetrachloroethylene 3433
Tetracycline 3026
Tetramethylenedisulfotetramine ... 1173, 1220
TETS, DFP 2105
Textile Dye 2860
Theacrine 1459
Theophylline 1665
Three-Dimensional Cell Culture 2179
Threshold of Toxicological Concern 2163
Thrombocytopenia 1657
Thrombogenesis 2440
Thymoma 2714
Thyroid Hormone 1892
Thyroid Peroxidase Inhibition 2222
TiO2 (Titanium Dioxide) 2778, 2791,2859
TiO2 Nanoparticles 1501
Titanium 2784
Titanium Dioxide 1519, 2543, 2801
TLR 1851
TMPD 1904
TNP 2160
Tobacco ... 1107, 1163, 2311, 2971, 3040, 3395
Tobacco Products 3396
Tobacco Smoke 2696, 3200
Tobacco Use 2983
Toluene 1201, 2229
Tolvaptan 3175, 3455
Topical Therapies 3083
Total Dissolved Solidsfenton's Reagent 1276
Total Particulate Matter, Cigarette Smoke 3038
Total Petroleum Hydrocarbon 1696
Tox21 ..... 1095, 2604
Toxcast 2608, 2640, 3300
Toxic Load Model 2170
Toxicant Screening Platform 3388
Toxicarnival 1381
Toxicity; Acute 1112, 1246, 1430, 1439, 1471, 1541, 1576, 1626, 1632, 1657, 1666, 1795, 1798, 1963, 1975, 1986, 1994, 2015, 2192, 2460, 2470, 2484, 2485, 2794, 2808, 2848, 2927, 2966, 2990, 3007, 3008, 3028, 3062, 3064, 3072, 3138, 3185, 3186, 3397, 3431, 3491
Toxicity; Chronic 1079, 1225, 1267, 1290, 1466, 1471, 1511, 1544, 1547, 1620, 1632, 1633, 1649, 1676, 1744, 1896, 1965, 2015, 2019, 2173, 2182, 2247, 2331, 2367, 2401, 2402, 2537, 2613, 2679, 2688, 2795, 2846, 2850, 2883, 2926, 3007, 3081, 3096, 3097, 3138, 3182, 3209, 3284, 3427
Toxicogenomics 1072, 1108, 1119, 1120, 1165, 1270, 1480, 1802, 2088, 2164, 2393, 2432, 2455, 2546, 2607, 2610, 2612, 2613, 2614, 2616, 2686, 2729, 2771, 2807, 2934, 2946, 3018, 3029, 3050, 3140, 3175, 3188, 3409, 3468, 3484
Toxicokinetics 1146, 1454, 1461, 1620, 1634, 1655, 2043, 2216, 2223, 2225, 2237, 2458, 2541, 2656, 2674, 2893, 2908, 3112, 3403, 3433, 3437, 3441, 3442, 3446, 3448, 3532
Toxicomethylomics 3219
Toxicoproteomics 3490
Toxprint 2709
TPN902 1652
Traffic-Related Air Pollution 1382
Transcription Factors 1257, 1537, 1564, 1738, 1739, 1958, 2054, 2066, 2074, 2292, 2298, 2299, 2301, 2307, 2311, 2335, 2357, 2423, 2438, 2601, 2624, 3017, 3270
Transcriptome 2247
Transcutaneous Exposure 2806
Transforming Growth Factor β1 2879
Transgender 2233
Transgenic Models 1587, 1799, 1939, 2033, 2063, 2356, 2374, 2904, 3094, 3102, 3104
Transient Receptor Potential 1285, 2244
Transport 3120
Transporters 1635, 2371
Trastuzamab 2481
Travel Abroad 1687
Tremolite 2672
Tributyltin 1883, 2545, 3264
Trichloroethylene 1196, 2729, 3198, 3434
Triclosan 1058, 1798, 1802, 2153, 2332, 2676
Tricresyl Phosphate 1231
Triethylene Glycol 2270
Triphenyl Phosphate 1850
Triptolide 1482
Troglitazone 1073, 1918, 1995, 1999
Troponin 2824
Trovafloxacin 1334
TRP Channels 1573
TRPA1 2251
TRPV4 antagonists 1301
Trypan Blue Staining 2763
Tryptophan Catabolite, Endogenous Agonist, Rna Seq 2427
TTC 2475
Tuberculosis 1495, 1978
Tumor Necrosis Factor Alpha 2878
Tungsten 1554, 1555
Tungsten Oxide 2798
Tympanotonus Fuscatus 1277
Type 2 Diabetes 1850, 2487
Type 2 Diabetes Mellitus 2620
Tyrosine Kinase Inhibitor 1650, 2488
Ultrafine Particles 1168
Undergraduate Student 1383, 1447, 1524, 1550, 1697, 1813, 1880, 1895, 2059, 2256, 2257, 2982, 3293, 3348, 3501
Unfolded Protein Response 2291
Unilateral Ureteral Obstruction 3133
Uranium 1560, 1691
Vaccine 1624
Valproate 3263
Valproic Acid 2003
Vanadium 1557, 3002
Vapor Intrusion 2691
Vascular Adhesion Molecule-1 2821
Vascular Dysfunction 2247
Vascular Injury 2849
Vascular Smooth Muscle Cells, ... 2177
Vasopressin 2835
VDR 1882
Vernonia Amygdalina 1327
Vesicants 1600
Vietnamese Women 1143
Vincristine 2151
Viral 2811
Virtual Screening 2652
Vitamin A 2282
Vitamin D 1307, 1882, 2968
Vitamin E 3245
Vitamin E Succinate 2333
Volatile Organic Compounds 1199, 1201, 1204, 1406, 1453, 1686, 1689, 1984, 2132, 2460, 2621, 2661, 2678, 2691, 2702, 2972, 2993, 3046, 3198, 3216, 3219, 3241
VX 2101, 2107, 2117
Weight of Evidence 2668, 2703, 2709, 2884
Welding Fume 1289
Wheat 2276
Whole-Body and Nose-Only Inhalation 3425
Wildfire 2266
Wine 1264
Wnt/β-catenin 1964
Wnt-Beta Catenin Signaling 2025
Women in Science 1380
Women with Secondary Infertility 2735
World Trade Center 2247
Wound Healing 3516
Xenobiotic Metabolism 2294
Xenobiotic Metabolizing Enzymes 2594
Xenobiotic Processing Genes 3144
Xenobiotic Transporters 1221, 1476, 1635, 1822, 1885, 1985, 2316, 2731, 3053, 3112, 3114, 3116, 3226, 3232, 3233, 3234, 3240, 3272, 3345, 3413, 3431, 3432
XRF 1545
Yarrowia Lipolytica 3004
Zearalenone 1140, 2276, 3021
Zeaxanthin 3004
Zebrafish 1175, 1185, 1239, 1588, 1793, 1795, 1798, 1802, 1805, 2095, 2456, 2947, 2950, 3136, 3296
Zebrafish Liver 2594
Zebrafish, Neuromast, Metastasis 1807
Zebrafish, Skeleton 1794
Zidovudine 1621
Zinc 1102, 1274, 1598, 2903, 3125, 3223
Zinc Dimethyldithiocarbamate (Ziram) 2892
Zinc Sulfate 2948
Ziram 2339
ZMapp 1626
Zuotai 1572

Notes

Notes

Toxicological Sciences

5-YEAR IMPACT FACTOR

Ranked Among the Top Journals in Toxicology

UNDER 2 WEEKS

Rapid Online Publication

Optional Open Access

The mission of *ToxSci*, the official journal of the Society of Toxicology, is to publish the most influential research in the field of toxicology.

Meet with Editor-in-Chief Gary W. Miller
SOT Pavilion, Booth 500
Monday to Wednesday
10:00 am–11:00 am and 2:00 pm–3:00 pm

OXFORD UNIVERSITY PRESS

OXFORD OPEN

SOT | Society of Toxicology

55th Annual Meeting and ToxExpo™

March 13–17, 2016

2016 • New Orleans

SOT

Annual Meeting & ToxExpo

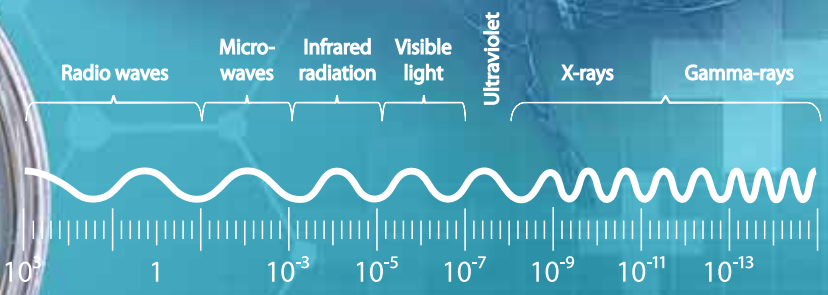
24
chromium
Cr
51.996

33
arsenic
As
74.9216

80
mercury
Hg
200.59

82
lead
Pb
207.2

86
radon
Rn
222.0176



Society of Toxicology

1821 Michael Faraday Drive, Suite 300 • Reston, VA 20190

T: 703.438.3115 • F: 703.438.3113 • Email: sothq@toxicology.org

www.toxicology.org

FSC © Mixed Sources
Product group from well-managed forests, controlled sources and recycled wood or fiber
www.fsc.org Cert no. XXX-XXX-XXXX
© 1996 Forest Stewardship Council

We are proud to print this publication entirely on Forest Stewardship Council certified paper. FSC certification ensures that the paper in this publication contains fiber from well-managed and responsibly harvested forests that meet strict environmental, social, and economic standards.